



Short communication

Hydrocortisone and dexamethasone dose-dependently stabilize mast cells derived from rat peritoneum



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ABSTRACT

Background: Besides their anti-inflammatory properties, corticosteroid drugs exert anti-allergic effects. Exocytosis of mast cells is electrophysiologically detected as the increase in the whole-cell membrane capacitance (Cm). Therefore, the lack of such increase after exposure to the drugs suggests their mast cell-stabilizing effects.

Methods: We examined the effects of 1, 10, 100 and 200 μ M hydrocortisone or dexamethasone on the degranulation from rat peritoneal mast cells. Employing the whole-cell patch-clamp recording technique, we also tested their effects on the Cm during exocytosis.

Results: At relatively lower concentrations (1, 10 μ M), both hydrocortisone and dexamethasone did not significantly affect the degranulation from mast cells and the increase in the Cm induced by GTP- γ -S. Nevertheless, at higher doses (100, 200 μ M), these drugs inhibited the degranulation from mast cells and markedly suppressed the GTP- γ -S-induced increase in the Cm.

Conclusions: Our results provided electrophysiological evidence for the first time that corticosteroid drugs, such as hydrocortisone and dexamethasone, inhibited the exocytotic process of mast cells in a dose-dependent manner. The mast cell-stabilizing effects of these drugs may be attributable to their “non-genomic” action, by which they exert rapid anti-allergic effects.

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Introduction

Corticosteroid drugs are most commonly used to treat acute or chronic inflammatory diseases, including arthritis, dermatitis and many types of autoimmune disorders including systemic lupus erythematosus and inflammatory bowel disease [1]. These drugs usually exert their anti-inflammatory properties by binding to the cytosolic glucocorticoid receptors and suppressing the gene expression necessary for the activation and proliferation of T-lymphocytes [2]. In allergic disorders, including bronchial asthma and anaphylaxis, corticosteroids are commonly used to improve the protracted symptoms or to prevent the recurrence of episodes [3,4]. Concerning the mechanisms, these drugs suppress the late-phase reaction of the diseases, in which the over-activation of T-lymphocytes or eosinophils is primarily responsible

for the pathogenesis [3,4]. However, previous *in vivo* studies also revealed that corticosteroids can ameliorate the acute-phase reaction of the allergic diseases [5,6], for which the release by mast cells of chemical mediators is mainly involved in the pathogenesis [7]. These findings strongly suggest that corticosteroids have anti-allergic properties, although we know little about the mechanisms. Measuring the amount of chemical mediators secreted from mast cells, including histamine, leukotrienes and prostaglandins, several studies tried to determine the effects of glucocorticoids, such as dexamethasone, on the stabilization of mast cells [8,9]. However, no one has directly examined the effects of these drugs on the process of exocytosis itself in mast cells. In our recent studies, detecting the changes in whole-cell membrane capacitance (Cm) in mast cells, we demonstrated that anti-allergic or anti-microbial drugs, including olopatadine, ketotifen and clarithromycin, directly inhibited the exocytotic process, and thus exerted mast cell-stabilizing effects [10–12]. In the present study, applying this whole-cell patch-clamp recording technique to mast cells obtained from rat peritoneum, we tested the effects of corticosteroid drugs, such as hydrocortisone and dexamethasone,

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on the changes in the Cm to examine their ability to stabilize mast cells. Here, this study provides electrophysiological evidence for the first time that hydrocortisone and dexamethasone inhibit the exocytotic process of mast cells in a dose-dependent manner. The study also shows that the mast cell-stabilizing effects of these drugs are ascribable to their “non-genomic” action, by which they exert rapid anti-allergic effects.

Materials and methods

Degranulation of mast cells

We isolated mast cells from Male Wistar rat peritoneum (Japan SLC Inc., Shizuoka, Japan) as described previously [10–12]. Briefly, we washed the peritoneal cavity with standard external (bathing)

solution and obtained mast cell suspension. Hydrocortisone, purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan), and dexamethasone, from Wako Pure Chem Ind. (Osaka, Japan), were dissolved in the bathing solution (final concentrations; 1, 10, 100 and 200 μM , respectively). After incubating mast cells for 10 min in each solution, we externally induced exocytosis using compound 48/80 (Sigma Aldrich Co., St. Louis, MO, USA) [10–13]. As previously described [10–14], we counted the degranulating mast cells and calculated their ratio to all mast cells.

Membrane capacitance measurements

We scattered mast cells in the bathing solutions containing no drug and each concentration of either hydrocortisone or dexamethasone (1, 10, 100 and 200 μM). We conducted standard patch-

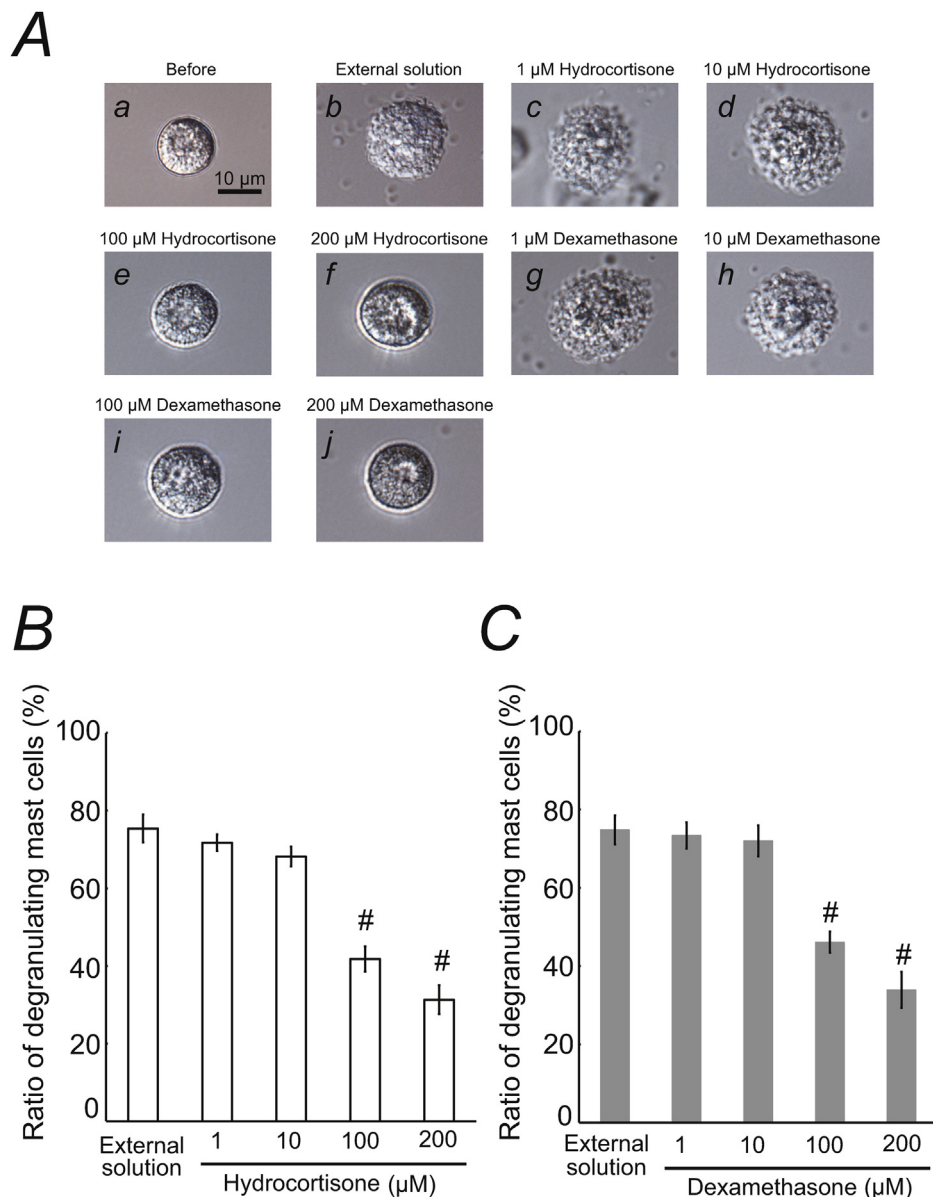


Fig. 1. Effects of corticosteroid drugs on mast cell degranulation. A: Differential-interference contrast (DIC) microscopic images were taken before (a) and after exocytosis was externally induced by compound 48/80 in mast cells incubated in the external solutions containing no drug (b), 1 μM hydrocortisone (c), 10 μM hydrocortisone (d), 100 μM hydrocortisone (e), 200 μM hydrocortisone (f), 1 μM dexamethasone (g), 10 μM dexamethasone (h), 100 μM dexamethasone (i) and 200 μM dexamethasone (j). Effects of different concentrations (1, 10, 100 and 200 μM) of hydrocortisone (B) and dexamethasone (C). After the mast cells were incubated in the external solutions containing no drug or either drug, exocytosis was induced by compound 48/80. The numbers of degranulating mast cells are expressed as percentages of the total mast cell numbers in selected bright fields. [#] $p < 0.05$ vs. incubation in the external solution alone. Values are means $\pm$ SEM ($n = 6$; 10 views from 6 of each condition). Differences were analyzed by ANOVA followed by Dunnett's or Student's t test.

clamp whole-cell recordings, using an EPC-9 patch-clamp amplifier system (HEKA Electronics, Lambrecht, Germany). As previously described [10–13], exocytosis was internally induced by adding 100 μ M guanosine 5'- α -(3-thiotriphosphate) (GTP- γ -S) (EMD Bioscience Inc., La Jolla, CA, USA) into the internal (patch-pipette) solution. For the measurement of the membrane capacitance, we used the Lock-in amplifier of an EPC-9 Pulse program as previously described [10–13]. In the whole-cell recording configuration, we continuously measured the membrane capacitance (C_m), together with the membrane conductance (G_m) and series conductance (G_s).

Statistic analyses

We analyzed data by PulseFit software (HEKA Electronics, Lambrecht, Germany) and reported as means $\pm$ SEM. A value of $p < 0.05$ was considered to be significant.

Results and discussion

Concentrations of hydrocortisone and dexamethasone used in the degranulation study

In humans, the serum concentration of hydrocortisone reaches around 1 and 2 μ M when physiological doses are orally administered [15]. However, according to *in vitro* studies using rat basophilic leukemia cells, concentrations as high as 10–100 μ M corticosterone was necessary to effectively elicit its inhibitory properties on the release of histamine [16]. Therefore, in our study, we tried hydrocortisone at doses starting from 1 μ M up to 200 μ M (Fig. 1).

Dexamethasone is one of the synthetic members of corticosteroid drugs and is chemically based on the structure of hydrocortisone [17]. However, compared to other corticosteroids, such as hydrocortisone or corticosterone, dexamethasone has

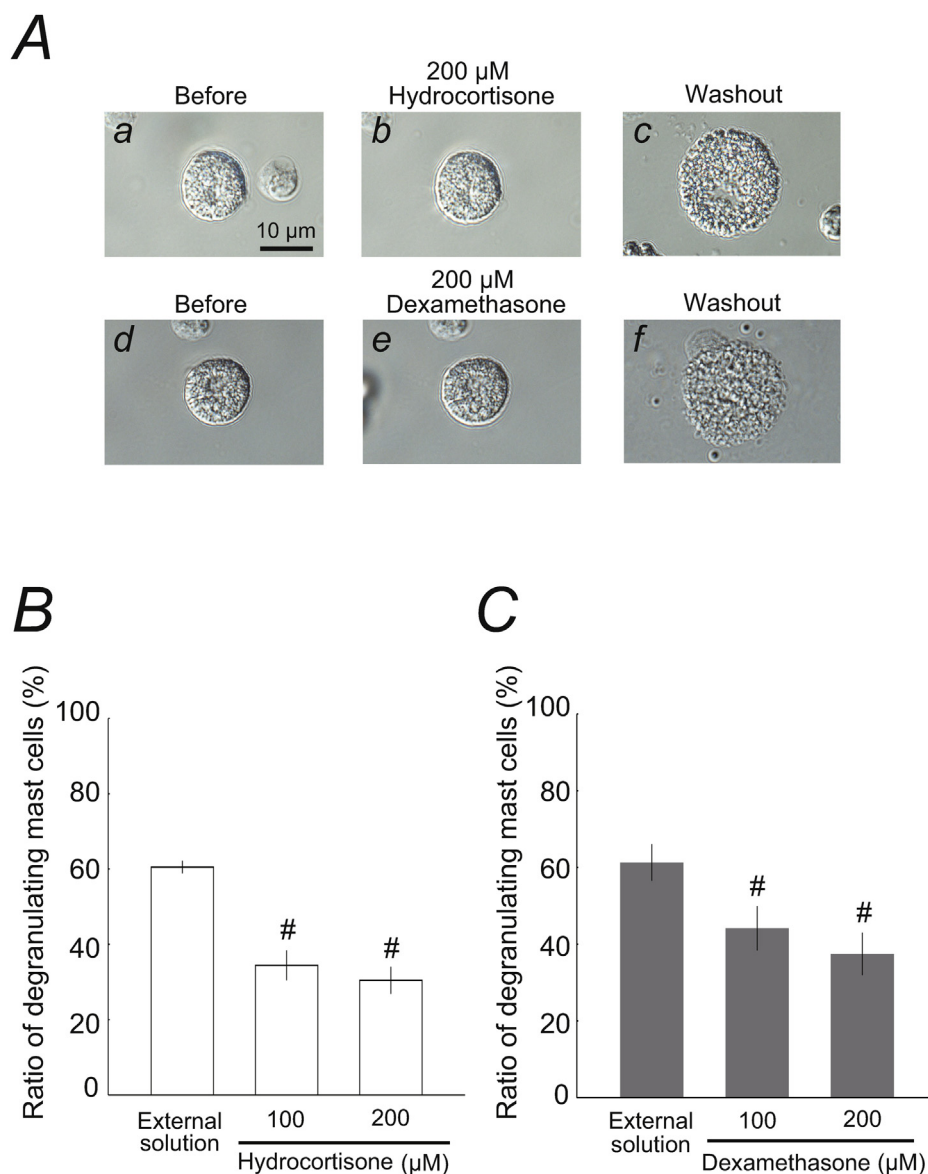


Fig. 2. Reversibility and pre-incubation time dependency of corticosteroid effects. (A) Differential-interference contrast (DIC) microscopic images were taken before (a, d) and after exocytosis was externally induced by compound 48/80 (b, c, e, f). Mast cells incubated in 200 μ M hydrocortisone (b) or dexamethasone (e) were washed thoroughly with the external solution (c, f) before exocytosis was induced. Effects of high concentrations (100 and 200 μ M) of hydrocortisone (B) and dexamethasone (C) with shorter period of pre-incubation time. After mast cells were incubated in the external solutions containing no drug or either drug for 3 min, exocytosis was induced by compound 48/80. The numbers of degranulating mast cells are expressed as percentages of the total mast cell numbers in selected bright fields. [#] $p < 0.05$ vs. incubation in the external solution alone. Values are means $\pm$ SEM ($n = 6$; 10 views from 6 of each condition). Differences were analyzed by ANOVA followed by Dunnett's or Student's t test.

much higher potency as a glucocorticoid [18]. Therefore, we additionally tested the effects of dexamethasone on the mast cell degranulation (Fig. 1). In previous *in vivo* studies, the plasma concentration of dexamethasone was less than 500 nM in humans when orally administered at physiological doses [19]. However, according to *in vitro* studies of cultured mast cells, concentrations as high as 1 μ M dexamethasone were required to decrease their numbers by suppressing their survival [20]. Additionally, in mast cells isolated from human lung or skin, much higher doses of dexamethasone were necessary to more effectively elicit its suppressive property for the histamine release [8,9]. Therefore, in the present study, we tried dexamethasone at doses starting from 1 μ M up to 200 μ M (Fig. 1).

Effects of hydrocortisone and dexamethasone on degranulation of rat peritoneal mast cells

Mast cells incubated in the external solution with compound 48/80 (10 μ g/ml) showed more wrinkles on their cell surface than those incubated without the compound (Fig. 1Ab vs. a). Additionally, they released secretory granules (degranulation) due to exocytosis (Fig. 1Ab vs. a). Mast cells that were pre-incubated in relatively lower doses of hydrocortisone (1, 10 μ M; Fig. 1Ac, d) or dexamethasone (1, 10 μ M; Fig. 1Ag, h) resembled those pre-incubated in the external bathing solution alone (Fig. 1Ab). However, most of the cells pre-incubated in relatively higher doses of hydrocortisone (100, 200 μ M; Fig. 1Ae, f) or dexamethasone (100, 200 μ M; Fig. 1Ai, j) failed to show such findings of exocytosis.

To quantitatively determine the effects of hydrocortisone and dexamethasone, we then counted the numbers of degranulating mast cells and calculated their ratio to all mast cells (Fig. 1B and C). In the absence of corticosteroid drugs, compound 48/80 caused degranulation in $75.4 \pm 3.6\%$ of the entire mast cells ($n = 6$; Fig. 1B). Relatively lower concentrations of hydrocortisone (1, 10 μ M), did not significantly alter the percentages of degranulating mast cells (Fig. 1B). However, higher concentrations (100, 200 μ M), remarkably reduced the numbers of degranulating mast cells (100 μ M, $41.8 \pm 3.3\%$; 200 μ M, $31.3 \pm 3.7\%$; $n = 6$, $p < 0.05$; Fig. 1B). Relatively lower concentrations of dexamethasone (1, 10 μ M) did not significantly alter the percentages of degranulating mast cells (Fig. 1C). However, similarly to hydrocortisone (Fig. 1B), higher concentrations of dexamethasone (100, 200 μ M), remarkably reduced the numbers of degranulating mast cells (100 μ M, $46.1 \pm 2.7\%$; 200 μ M, $33.9 \pm 4.6\%$; $n = 6$, $p < 0.05$; Fig. 1C). Therefore, consistent with our previous findings demonstrated with anti-allergic drugs or macrolide antibiotics [10–12], corticosteroid drugs, such as hydrocortisone and dexamethasone, inhibited the degranulation of rat peritoneal mast cells dose-dependently.

Corticosteroid drugs usually exert anti-inflammatory properties by suppressing the proliferation of T-lymphocytes and reducing their synthesis of pro-inflammatory cytokines [1]. Such mechanisms are ascribable to the “genomic” action of corticosteroids, which is initiated by their binding to the cytosolic glucocorticoid receptors [2,21]. Subsequently, the newly formed complexes translocate themselves into the cell nucleus, where the drugs bind to glucocorticoid response elements in the promoter region of the target genes. This results in the transcriptional repression of the genes responsible for lymphocyte proliferation or their protein synthesis. In previous *in vivo* studies, the continuous administration of glucocorticoids ameliorated acute allergic symptoms, such as skin weal and flare [22]. Since long-term exposure to these drugs actually decreased the numbers of mast cells in inflamed tissues [23,24], it was likely to be attributable to their genomic action. In our study, using rat peritoneal mast cells, we additionally demonstrated that the short-term exposure to

corticosteroids also inhibits the process of exocytosis directly. These results strongly suggested the involvement of “non-genomic” mechanisms in the effects of corticosteroids, independent of targeting the gene expression.

Reversibility and pre-incubation time dependency of corticosteroid effects

To examine whether the effects of corticosteroids on the degranulation of mast cells are reversible, mast cells pre-incubated in the highest concentrations of hydrocortisone or dexamethasone (200 μ M) were washed thoroughly with the external solutions. Then exocytosis was externally induced by compound 48/80. Mast cells pre-incubated in either drug failed to show any findings of exocytosis (Fig. 2Ab vs. a, e vs. d). However, after the washout, they released secretory granules in response to the compound stimuli (Fig. 2Ac and f), indicating that the effects of corticosteroids on the degranulation of mast cells was reversible.

To examine whether such effects of the corticosteroids are dependent on the pre-incubation time with these drugs, we incubated mast cells for a shorter period of time (3 min) in hydrocortisone or dexamethasone containing external solutions

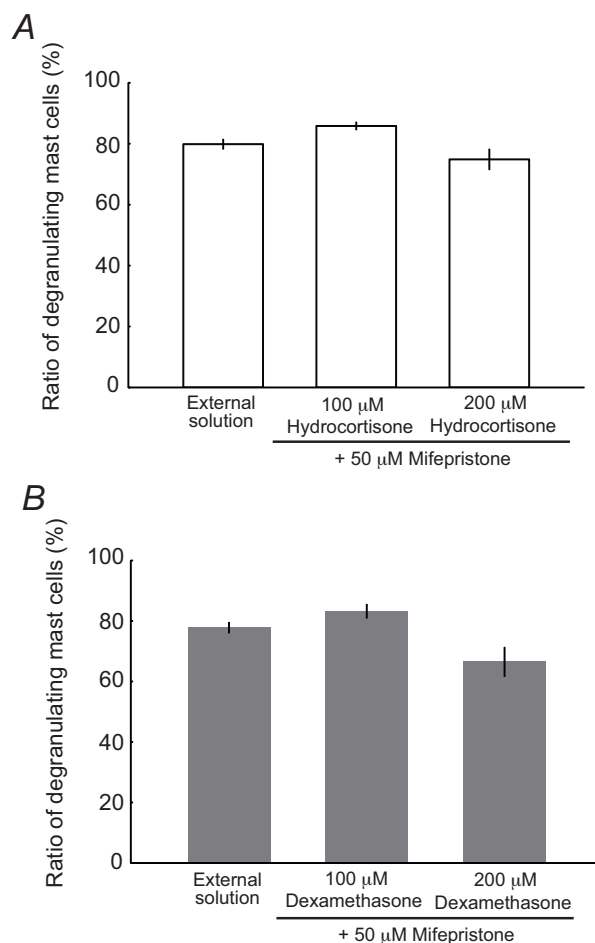


Fig. 3. Effects of mifepristone on mast cell degranulation. Effects of high concentrations (100 and 200 μ M) of hydrocortisone (A) and dexamethasone (B) in the presence of a glucocorticoid receptor antagonist. After mast cells were incubated in the external solutions containing no drug or either drug in the presence of 50 μ M mifepristone, exocytosis was induced by compound 48/80. The numbers of degranulating mast cells are expressed as percentages of the total mast cell numbers in selected bright fields. Values are means $\pm$ SEM ($n = 6$; 10 views from 6 of each condition). Differences were analyzed by ANOVA followed by Dunnett's or Student's *t* test.

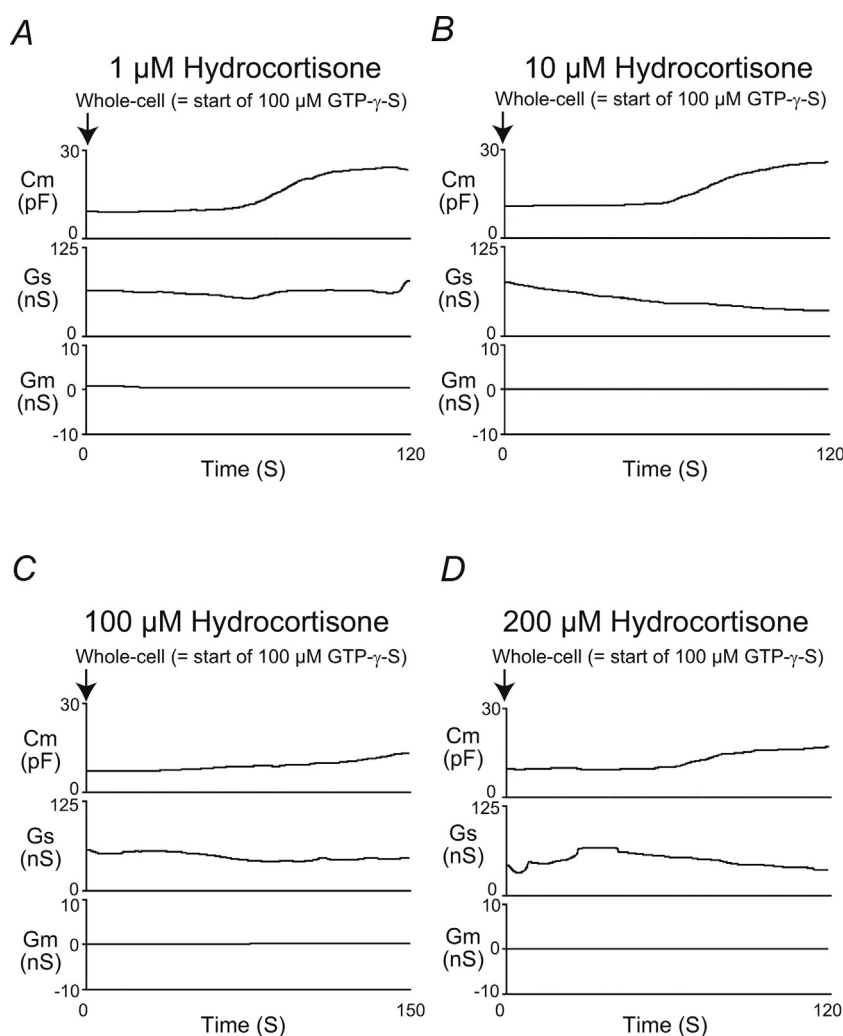


Fig. 4. Hydrocortisone-induced changes in mast cell membrane capacitance, series and membrane conductance during exocytosis. After the mast cells were incubated in the external solutions containing 1 μM (A), 10 μM (B), 100 μM (C) or 200 μM hydrocortisone (D), the whole-cell recording configuration was established in single mast cells and dialysis with 100 μM GTP-γ-S was started. Membrane capacitance, series and membrane conductance were monitored for at least 90 s. *N* = 5 for each trace. Cm, membrane capacitance; Gs, series conductance; Gm, membrane conductance.

(100 and 200 μM). Then exocytosis was externally induced by compound 48/80. Similar to the effects obtained after a longer incubation period (10 min: Fig. 1B and C), higher concentrations of these corticosteroids (100, 200 μM), remarkably reduced the numbers of degranulating mast cells (Fig. 2B and C). These results indicated that mast cells responded quickly to the “non-genomic” mechanisms of corticosteroids, by which they inhibited the process of exocytosis.

Effects of a glucocorticoid receptor antagonist, mifepristone, on degranulation of mast cells

To reveal whether the effects of corticosteroids on the degranulation of mast cells are mediated through the glucocorticoid receptors, we examined the effects of hydrocortisone and dexamethasone in the presence of mifepristone (RU-486) (Cayman Chemical, Ann Arbor, MI, USA), a competitive antagonist of glucocorticoid receptors [25,26]. Mast cells were incubated in hydrocortisone or dexamethasone containing external solutions (100 and 200 μM) in the presence of mifepristone. Since mifepristone was not dissolved in the external solution at concentrations higher than 100 μM, we tried 50 μM in the present study. Then exocytosis was induced externally by compound 48/80.

In the absence of mifepristone, both hydrocortisone and dexamethasone (100 and 200 μM) remarkably reduced the numbers of degranulating mast cells (Fig. 1B and C). However, in the presence of mifepristone, these drugs did not significantly alter the percentages of degranulating mast cells (Fig. 3A and B). These results indicated that the suppressive effects of corticosteroids on the degranulation of mast cells were dependent on the glucocorticoid receptors.

Effects of hydrocortisone and dexamethasone on membrane capacitance of rat peritoneal mast cells

In our previous studies, microscopic changes in megakaryocyte or lymphocyte membranes were accurately monitored by measuring the whole-cell membrane capacitance (Cm) [27–34]. In mast cells, the process of degranulation during exocytosis is successively monitored by the increase in the Cm [10–13,35,36]. Hence, in our study, to examine the effects of hydrocortisone and dexamethasone on the process of exocytosis, we pre-incubated mast cells in hydrocortisone- or dexamethasone- containing external solutions and measured the changes in Cm (Figs. 4 and 5). We showed the effects of 1, 10, 100 and 200 μM hydrocortisone or dexamethasone on the Cm, Gs and Gm in each figure. Table 1 summarized the

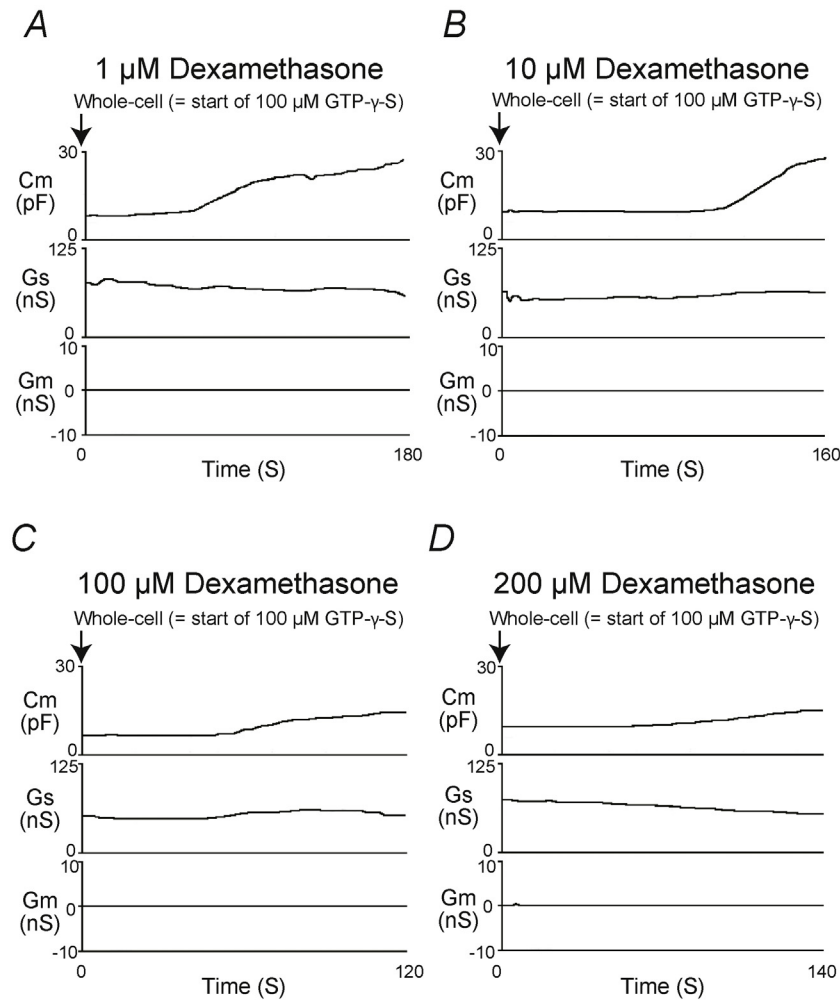


Fig. 5. Dexamethasone-induced changes in mast cell membrane capacitance, series and membrane conductance during exocytosis. After the mast cells were incubated in the external solutions containing 1 μ M (A), 10 μ M (B), 100 μ M (C) or 200 μ M dexamethasone (D), the whole-cell recording configuration was established in single mast cells and dialysis with 100 μ M GTP- γ -S was started. Membrane capacitance, series and membrane conductance were monitored for at least 90 s. $N = 5$ for each trace. Cm, membrane capacitance; Gs, series conductance; Gm, membrane conductance.

Table 1

Summary of changes in membrane capacitance (Cm) in different concentrations of corticosteroid-containing solutions.

Agents	N	Cm before GTP- γ -S Internalization (pF)	Cm after GTP- γ -S Internalization (pF)	Δ Cm (pF)
External solution (control)	5	9.78 ± 0.61	26.5 ± 2.06	16.7 ± 1.99
1 μ M Hydrocortisone	5	9.23 ± 0.57	25.4 ± 1.50	15.3 ± 0.80
10 μ M Hydrocortisone	5	9.74 ± 0.55	25.9 ± 2.50	16.2 ± 2.73
100 μ M Hydrocortisone	5	8.26 ± 0.42	16.1 ± 2.91	$7.81 \pm 2.72^*$
200 μ M Hydrocortisone	5	9.62 ± 0.51	18.8 ± 0.56	$6.71 \pm 1.70^*$
1 μ M Dexamethasone	5	9.34 ± 0.26	26.8 ± 2.05	17.5 ± 1.93
10 μ M Dexamethasone	5	9.06 ± 0.50	25.4 ± 1.16	16.4 ± 0.77
100 μ M Dexamethasone	5	7.25 ± 0.55	16.7 ± 1.58	$9.46 \pm 1.20^*$
200 μ M Dexamethasone	5	8.80 ± 0.48	15.1 ± 2.18	$6.34 \pm 2.43^*$

Values are means $\pm$ SEM. Cm = membrane capacitance.

* $p < 0.05$ vs. Δ Cm in external solution (control).

changes in the Cm. As previously demonstrated [10–13,37,38], the internal addition of GTP- γ -S to mast cells almost tripled the value of Cm (from 9.78 ± 0.61 to 26.5 ± 2.06 pF, $n = 5$, $p < 0.05$; Table 1).

When mast cells were pre-incubated in lower doses of hydrocortisone (1, 10 μ M), the increase in the Cm was almost identical to that of the mast cells pre-incubated in the bathing solution without drugs (Fig. 4A and B, Table 1). However, higher doses of hydrocortisone (100, 200 μ M) significantly inhibited the GTP- γ -S-induced increase in the Cm (Fig. 4C and D, Table 1).

Similarly to hydrocortisone, the lower doses of dexamethasone (1, 10 μ M) did not alter the increase in the Cm (Fig. 5A and B, Table 1), which was almost identical to that of the external solution alone as demonstrated in our previous studies [10–13]. However, pre-incubation in higher doses of dexamethasone (100, 200 μ M) markedly halted the increase in the Cm (Fig. 5C and D, Table 1). The results provided electrophysiological evidence for the first time that both hydrocortisone and dexamethasone inhibited the exocytotic process of mast cells dose-dependently. This strongly supported our findings that were demonstrated in Fig. 1.

According to previous patch-clamp studies, the process degranulation in mast cells during exocytosis is successively monitored by the gradual increase in the whole-cell C_m [13,37,39]. Employing this electrophysiological approach, our recent studies demonstrated the inhibitory effects of anti-allergic drugs or macrolide antibiotics on the exocytotic process of mast cells [10–12]. In these studies, the mast cell-stabilizing effects of the drugs were quantitatively assessed by the degree of suppression in the increase in the C_m . In the present study, using such an approach, we provide direct evidence for the first time that corticosteroids, such as hydrocortisone and dexamethasone, dose-dependently inhibited the exocytotic process, and thus exerted mast cell-stabilizing effects. Since corticosteroid drugs are highly lipophilic [40], they were thought to easily penetrate into the cytoplasm of mast cells through the plasma membranes [41]. This may allow the drugs to bind to the cytosolic glucocorticoid receptors, finally repressing the gene expression responsible for the mast cell growth or survival [42,43]. Besides their penetration into the cytoplasm and the subsequent accumulation there, corticosteroids are also known to be incorporated into the lipid bilayers within the plasma membranes [21]. Additionally, a recent morphological study revealed the localization of glucocorticoid receptors within the plasma membrane of mast cells [44]. In contrast to the cytosolic glucocorticoid receptors that exert biological responses by regulating the gene expression, the membrane-localized glucocorticoid receptors directly influence the intracellular Ca^{2+} signaling pathway by coupling to G proteins or functionally modulating the Ca^{2+} channels [45,46]. In mast cells, as we recently demonstrated in a patch-clamp study, an increase in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) primarily triggers exocytosis [11]. Therefore, in the present study, in addition to the involvement of membrane proteins that have been advocated [43], the membrane receptor-mediated changes in the $[Ca^{2+}]_i$ may have contributed to the non-genomic mechanisms of the corticosteroids, by which they exerted mast cell-stabilizing properties. In mast cells, compound 48/80, a G protein stimulator, directly activates membrane GTPase to stimulate the phospholipase C (PLC)-mediated signaling pathway [47]. Since the PLC activity was dose-dependently suppressed by adrenal glucocorticoids [48], such pharmacological interaction between the compound and the corticosteroids may have also contributed to their mast cell-stabilizing properties in the present study.

In summary, this study provided electrophysiological evidence for the first time that corticosteroid drugs, such as hydrocortisone and dexamethasone, dose-dependently inhibited the exocytotic process of mast cells, indicating their usefulness as mast cell-stabilizers. Such mast cell-stabilizing effects of these drugs were ascribable to their “non-genomic” action, by which they exert rapid anti-allergic effects.

Declaration of interest

The authors declare that there is no conflict of interest.

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References

- [1] Rhen T, Cidlowski JA. Antiinflammatory action of glucocorticoids—new mechanisms for old drugs. *N Engl J Med* 2005;353(16):1711–23.
- [2] Lu NZ, Wardell SE, Burnstein KL, Defranco D, Fuller PJ, Giguere V, et al. International union of pharmacology. LXV. The pharmacology and classification of the nuclear receptor superfamily: glucocorticoid, mineralocorticoid, progesterone, and androgen receptors. *Pharmacol Rev* 2006;58(4):782–97.
- [3] Barnes PJ, Adcock IM. How do corticosteroids work in asthma? *Ann Intern Med* 2003;139(5 (Pt 1)):359–70.
- [4] Cheng A. Emergency treatment of anaphylaxis in infants and children. *Paediatr Child Health* 2011;16(1):35–40.
- [5] Zhou J, Kang ZM, Xie QM, Liu C, Lou SJ, Chen YZ, et al. Rapid nongenomic effects of glucocorticoids on allergic asthma reaction in the guinea pig. *J Endocrinol* 2003;177(1):R1–4.
- [6] Hashimoto M, Shinozaki Y, Katori M. Immediate inhibitory effect of methylprednisolone sultepanate (U-67590A) on antigen-induced cutaneous and airway anaphylactic responses in guinea-pigs. *Br J Pharmacol* 1993;108(3):604–12.
- [7] Liu MC, Hubbard WC, Proud D, Stealey BA, Galli SJ, Kagey-Sobotka A, et al. Immediate and late inflammatory responses to ragweed antigen challenge of the peripheral airways in allergic asthmatics: cellular, mediator, and permeability changes. *Am Rev Respir Dis* 1991;144(1):51–8.
- [8] Cohan VL, Udem BJ, Fox CC, Adkinson Jr. NF, Lichtenstein LM, Schleimer RP. Dexamethasone does not inhibit the release of mediators from human mast cells residing in airway, intestine, or skin. *Am Rev Respir Dis* 1989;140(4):951–4.
- [9] Schleimer RP, Schulman ES, MacGlashan Jr. DW, Peters SP, Hayes EC, Adams 3rd GK, et al. Effects of dexamethasone on mediator release from human lung fragments and purified human lung mast cells. *J Clin Invest* 1983;71(6):1830–5.
- [10] Baba A, Tachi M, Maruyama Y, Kazama I. Olopatadine inhibits exocytosis in rat peritoneal mast cells by counteracting membrane surface deformation. *Cell Physiol Biochem* 2015;35(1):386–96.
- [11] Baba A, Tachi M, Ejima Y, Endo Y, Toyama H, Matsubara M, et al. Anti-allergic drugs tranilast and ketotifen dose-dependently exert mast cell-stabilizing properties. *Cell Physiol Biochem* 2016;38:15–27.
- [12] Kazama I, Saito K, Baba A, Mori T, Abe N, Endo Y, et al. Clarithromycin dose-dependently stabilizes rat peritoneal mast cells. *Chemotherapy* 2016;61(6):295–303.
- [13] Kazama I, Maruyama Y, Takahashi S, Kokumai T. Amphipaths differentially modulate membrane surface deformation in rat peritoneal mast cells during exocytosis. *Cell Physiol Biochem* 2016;38:592–600.
- [14] Shimoda T, Liang Z, Suzuki H, Kawana S. Inhibitory effects of antipsychotic and anxiolytic agents on stress-induced degranulation of mouse dermal mast cells. *Clin Exp Dermatol* 2010;35(5):531–6.
- [15] Jung C, Greco S, Nguyen HH, Ho JT, Lewis JG, Torpy DJ, et al. Plasma, salivary and urinary cortisol levels following physiological and stress doses of hydrocortisone in normal volunteers. *BMC Endocr Disord* 2014;14:91.
- [16] Zhou J, Liu DF, Liu C, Kang ZM, Shen XH, Chen YZ, et al. Glucocorticoids inhibit degranulation of mast cells in allergic asthma via nongenomic mechanism. *Allergy* 2008;63(9):1177–85.
- [17] Xiao KP, Xiong Y, Rustum AM. Quantitation of trace betamethasone or dexamethasone in dexamethasone or betamethasone active pharmaceutical ingredients by reversed-phase high-performance liquid chromatography. *J Chromatogr Sci* 2008;46(1):15–22.
- [18] Liu D, Ahmet A, Ward L, Krishnamoorthy P, Mandelcorn ED, Leigh R, et al. A practical guide to the monitoring and management of the complications of systemic corticosteroid therapy. *Allergy Asthma Clin Immunol* 2013;9(1):30.
- [19] Weijtens O, Schoemaker RC, Cohen AF, Romijn FP, Lentjes EG, van Rooij J, et al. Dexamethasone concentration in vitreous and serum after oral administration. *Am J Ophthalmol* 1998;125(5):673–9.
- [20] Yoshikawa H, Nakajima Y, Tasaka K. Glucocorticoid suppresses autocrine survival of mast cells by inhibiting IL-4 production and ICAM-1 expression. *J Immunol* 1999;162(10):6162–70.
- [21] Alangari AA. Genomic and non-genomic actions of glucocorticoids in asthma. *Ann Thorac Med* 2010;5(3):133–9.
- [22] Cole ZA, Clough GF, Church MK. Inhibition by glucocorticoids of the mast cell-dependent weal and flare response in human skin in vivo. *Br J Pharmacol* 2001;132(1):286–92.
- [23] Goldsmith P, McGarity B, Walls AF, Church MK, Millward-Sadler GH, Robertson DA. Corticosteroid treatment reduces mast cell numbers in inflammatory bowel disease. *Dig Dis Sci* 1990;35(11):1409–13.
- [24] James A, Gyllfors P, Henriksson E, Dahlen SE, Adner M, Nilsson G, et al. Corticosteroid treatment selectively decreases mast cells in the smooth muscle and epithelium of asthmatic bronchi. *Allergy* 2012;67(7):958–61.
- [25] Yu S, Yang X, Zhu Y, Xie F, Lu Y, Yu T, et al. Systems pharmacology of mifepristone (RU486) reveals its 47 hub targets and network: comprehensive analysis and pharmacological focus on FAK-Src-Paxillin complex. *Sci Rep* 2015;5:7830.
- [26] Fjellidal R, Moe BT, Orbo A, Sager G. MCF-7 cell apoptosis and cell cycle arrest: non-genomic effects of progesterone and mifepristone (RU-486). *Anticancer Res* 2010;30(12):4835–40.
- [27] Kazama I, Maruyama Y, Nakamichi S. Aspirin-induced microscopic surface changes stimulate thrombopoiesis in rat megakaryocytes. *Clin Appl Thromb Hemost* 2014;20(3):318–25.
- [28] Kazama I, Maruyama Y, Murata Y. Suppressive effects of nonsteroidal anti-inflammatory drugs diclofenac sodium, salicylate and indomethacin on delayed rectifier K^+ -channel currents in murine thymocytes. *Immunopharmacol Immunotoxicol* 2012;34(5):874–8.

- [29] Kazama I, Maruyama Y, Matsubara M. Benidipine persistently inhibits delayed rectifier K(+)–channel currents in murine thymocytes. *Immunopharmacol Immunotoxicol* 2013;35(1):28–33.
- [30] Kazama I, Maruyama Y. Differential effects of clarithromycin and azithromycin on delayed rectifier K(+)–channel currents in murine thymocytes. *Pharm Biol* 2013;51(6):760–5.
- [31] Kazama I, Baba A, Maruyama Y. HMG-CoA reductase inhibitors pravastatin, lovastatin and simvastatin suppress delayed rectifier K(+)–channel currents in murine thymocytes. *Pharmacol Rep* 2014;66(4):712–7.
- [32] Baba A, Tachi M, Maruyama Y, Kazama I. Suppressive effects of diltiazem and verapamil on delayed rectifier K+–Channel currents in murine thymocytes. *Pharmacol Rep* 2015;67:959–64.
- [33] Kazama I, Ejima Y, Endo Y, Toyama H, Matsubara M, Baba A, et al. Chlorpromazine-induced changes in membrane micro-architecture inhibit thrombopoiesis in rat megakaryocytes. *Biochim Biophys Acta* 2015;1848(11 (Pt A)):2805–12.
- [34] Kazama I, Baba A, Endo Y, Toyama H, Ejima Y, Matsubara M, et al. Salicylate inhibits thrombopoiesis in rat megakaryocytes by changing the membrane micro-architecture. *Cell Physiol Biochem* 2015;35(6):2371–82.
- [35] Fernandez JM, Neher E, Gomperts BD. Capacitance measurements reveal stepwise fusion events in degranulating mast cells. *Nature* 1984;312 (5993):453–5.
- [36] Lorenz D, Wiesner B, Zipper J, Winkler A, Krause E, Beyermann M, et al. Mechanism of peptide-induced mast cell degranulation. Translocation and patch-clamp studies. *J Gen Physiol* 1998;112(5):577–91.
- [37] Neher E. The influence of intracellular calcium concentration on degranulation of dialysed mast cells from rat peritoneum. *J Physiol* 1988;395: 193–214.
- [38] Penner R, Neher E. Secretory responses of rat peritoneal mast cells to high intracellular calcium. *FEBS Lett* 1988;226(2):307–13.
- [39] Penner R. Multiple signaling pathways control stimulus–secretion coupling in rat peritoneal mast cells. *Proc Natl Acad Sci U S A* 1988;85(24):9856–60.
- [40] Ponec M, Kempenaar J, Shroot B, Caron JC. Glucocorticoids: binding affinity and lipophilicity. *J Pharm Sci* 1986;75(10):973–5.
- [41] Baxter JD, Rousseau GG, Benson MC, Garcea RL, Ito J, Tomkins GM. Role of DNA and specific cytoplasmic receptors in glucocorticoid action. *Proc Natl Acad Sci U S A* 1972;69(7):1892–6.
- [42] Belvisi MG. Regulation of inflammatory cell function by corticosteroids. *Proc Am Thorac Soc* 2004;1(3):207–14.
- [43] Oppong E, Flink N, Cato AC. Molecular mechanisms of glucocorticoid action in mast cells. *Mol Cell Endocrinol* 2013;380(1–2):119–26.
- [44] Oppong E, Hedde PN, Sekula-Neuner S, Yang L, Brinkmann F, Dorlich RM, et al. Localization and dynamics of glucocorticoid receptor at the plasma membrane of activated mast cells. *Small* 2014;10(10):1991–8.
- [45] Norman AW, Mizwicki MT, Norman DP. Steroid-hormone rapid actions, membrane receptors and a conformational ensemble model. *Nat Rev Drug Discov* 2004;3(1):27–41.
- [46] Chen HC, Farese RV. Steroid hormones: interactions with membrane-bound receptors. *Curr Biol* 1999;9(13):R478–81.
- [47] Chahdi A, Fraundorfer PF, Beaven MA: compound 48/80 activates mast cell phospholipase D via heterotrimeric GTP-binding proteins. *J Pharmacol Exp Ther* 2000;292(1):122–30.
- [48] Dwivedi Y, Rizavi HS, Rao JS, Pandey GN. Modifications in the phosphoinositide signaling pathway by adrenal glucocorticoids in rat brain: focus on phosphoinositide-specific phospholipase C and inositol 1,4,5-trisphosphate. *J Pharmacol Exp Ther* 2000;295(1):244–54.