
Changes in intracellular cyclic adenosine monophosphate levels in peripheral blood leukocytes during immunotherapy with vespid venom

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Background: The allergen-induced release of mediators from basophils is known to be decreased after rush venom immunotherapy (VIT) compared with pretreatment values. A rise in the intracellular cyclic adenosine monophosphate (cAMP) level is known to inhibit mediator release.

Objectives: To determine changes in cAMP levels in peripheral blood leukocytes (PBLs) during rush VIT and to evaluate their relation to allergen-specific reactivity of basophils.

Methods: Ten patients allergic to vespid venom (VV) were investigated before rush VIT and after reaching the maintenance dose. Five VV-allergic patients not undergoing VIT served as controls. Patients' PBLs were incubated with VV, and allergen-induced histamine and leukotriene release from basophils was measured. Levels of cAMP were determined in PBLs and in plasma.

Results: Immediately after rush VIT, VV-induced histamine release ($P = .04$) and VV-induced leukotriene release ($P = .01$) were significantly reduced. Intracellular cAMP levels increased significantly ($P = .047$). However, 6 months after VIT, mediator responses in basophils were comparable with pre-VIT values. No significant changes were found in the control group.

Conclusions: An increase in intracellular cAMP levels might account for the decreased reactivity of basophils to allergen after 1 week of VIT. However, similar to the decreased mediator release after 1 week of VIT, this is not a long-term effect as values returned to baseline after 6 months.

Ann Allergy Asthma Immunol. 2007;98:281–285.

INTRODUCTION

Venom immunotherapy (VIT) is highly effective in protecting patients with Hymenoptera venom allergy from further systemic anaphylactic reactions to stings.^{1,2} It has been reported that protection is achieved early after reaching the maintenance dose by rush or even ultrarush VIT.³

Various immunologic effects occur during VIT: some (such as a decreased ratio of specific IgE to IgG4) are only demonstrable after a longer period of treatment, whereas others (such as decreased allergen-specific mediator release from effector cells such as basophils) are seen very early.^{4–6} Venom immunotherapy is also associated with the induction of interleukin 10 (IL-10) production by T cells,⁷ and it has been shown that this cytokine might also be responsible for the decreased mediator release from basophils after rush VIT.⁵

Decreased release of mediators from effector cells of anaphylaxis immediately after rush VIT probably accounts for the early onset of protection. Corresponding intracellular mechanisms have not yet been elucidated. Cyclic adenosine monophosphate (cAMP) metabolism has a key function in basophil and mast cell reactivity. It has been shown that compounds that lead to a sustained increase in intracellular cAMP concentrations simultaneously inhibit IgE-triggered mediator release from human lung mast cells and basophils.^{8,9}

The aims of this study are to determine changes in cAMP levels during rush VIT in Hymenoptera venom-allergic patients and to evaluate the relation of these changes to allergen-specific reactivity of basophils. Patients with this type of allergy can be characterized very well by means of history and standardized immunologic tests. Venoms for treatment are also well characterized, and there are established treatment schedules. Therefore, rush VIT is an appropriate model to analyze the rapid change in immunologic variables during initial immunotherapy.

METHODS

Study Population

Ten patients scheduled to receive vespid VIT were enrolled. All the patients had a history of a systemic anaphylactic reaction to a yellowjacket sting and sensitization to vespid

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Received for publication September 25, 2006.

Received in revised form December 13, 2006.

Accepted for publication in revised form December 16, 2006.

venom (VV), proved by a positive reaction to VV on skin testing and/or the presence of VV specific IgE in serum. Titrated skin prick tests with VV (Reless; ALK-Scherax, Hamburg, Germany) were positive in 3 patients at a concentration of 1 $\mu\text{g/mL}$, in 5 at 10 $\mu\text{g/mL}$, and in 2 at 100 $\mu\text{g/mL}$. Specific IgE antibodies to VV were measurable by means of CAP-FEIA (Phadia, Freiburg, Germany) in 9 patients (CAP class 0, 1 patient; CAP class 2, 4 patients; CAP class 3, 3 patients; and CAP class 6, 2 patients). Venom immunotherapy was performed according to a rush protocol with up to 5 injections per day (0.02, 0.04, 0.08, 0.2, 0.4, 0.8, 2, 4, 8, 10, 20, 40, 60, 80, and 100 μg of VV) (Reless). Within 5 days the maintenance dose was reached. All the patients had tolerated the rush VIT without systemic anaphylactic symptoms. Final maintenance VIT consisted of injections of 100 μg of VV every 4 weeks. Blood samples were collected immediately before the start of VIT and within 24 hours of reaching the maintenance dose. In addition, 5 patients had another blood sample collected 6 months later while they received their regular maintenance dose of 100 μg of VV.

Five patients with VV allergy who were diagnosed as described previously herein but were not yet treated with VIT served as controls; blood samples were collected twice at an interval of 1 week. Results of titrated skin prick tests with VV were positive in 1 patient at 10 $\mu\text{g/mL}$ and in 3 patients at 100 $\mu\text{g/mL}$, and 1 patient had a positive intradermal test reaction at 1 $\mu\text{g/mL}$ of VV. Specific IgE antibodies to VV were measurable in 3 patients (CAP class 0, 2 patients; CAP class 3, 2 patients; and CAP class 6, 1 patient). Blood sampling for study purposes was approved by the ethical committee of Ludwig-Maximilian University and after patient informed consent was obtained.

Histamine Release

The histamine content of basophils and the release of histamine from basophils on stimulation with VV were determined as described previously.¹⁰ In brief, peripheral blood leukocytes (PBLs) were prepared by means of repeated centrifugation and resuspension in buffer. Samples containing 500 μL of cell suspension were incubated for 40 minutes with 100 μL each of buffer (blank), buffer plus IL-3 (10 ng/mL) (Amersham, Braunschweig, Germany), IL-3 (10 ng/mL) plus VV (0.01, 0.1, 0.5, or 1 $\mu\text{g/mL}$) (Reless), or IL-3 (10 ng/mL) plus anti-IgE (10^{-2} mol/L) (DAKO A/S, Hamburg, Germany) (positive control). Tests were performed as duplicates. Supernatants were recovered by centrifugation, and the histamine content was measured spectrofluorometrically according to the method described by Siraganian¹¹ using an autoanalyzer (Braun & L  bbe, Norderstedt, Germany). Total histamine content was measured after complete cell lysis induced by the addition of 70% perchloric acid (Merck, Darmstadt, Germany) to the cell suspension. The histamine release induced by exposure to a stimulus was calculated as a percentage of the total histamine content after subtraction of the blank value (incubation with buffer containing IL-3). Results are given as the percentage of total histamine content.

Leukotriene Release

Cellular antigen stimulation test with enzyme-linked immunosorbent assay (B  hlmann Laboratories AG, Sch  nenbuch, Switzerland) was performed according to the manufacturer's instructions. In brief, dextran (Macrodex 6%; Schiwa, Glandorf, Germany) was added to 10 mL of heparinized venous blood, and erythrocytes underwent sedimentation for 90 minutes at room temperature. Leukocytes were then pelleted by centrifugation and resuspended in buffer containing IL-3 (10 ng/mL); 200 μL of cell suspension was incubated with 50 μL of buffer (background), 50 μL of anti-IgE, or 50 μL of VV at concentrations of 0.01, 0.1, 0.5, or 1 $\mu\text{g/mL}$ (Reless). Tests were performed as duplicates. After 40 minutes the cell suspensions were centrifuged, and the sulfidoleukotriene concentration in the supernatants was measured by means of enzyme-linked immunosorbent assay.

cAMP Measurement

For measurement of plasma cAMP levels blood samples were collected into EDTA-containing syringes. After centrifugation for 10 minutes (300 rpm at 4  C), cAMP levels in the supernatants were measured by means of a radioimmunoassay (cAMP [¹²⁵I] assay system dual range) (Amersham). For determination of intracellular cAMP concentration, PBLs were isolated as described for the histamine release test. Cell suspensions were adjusted to 5.0×10^6 cells/mL; 100 μL of the cell suspension was resuspended in 400 μL of buffer containing the unspecific phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine and ascorbic acid. After incubation for 10 minutes at 37  C the preparation was boiled for 5 minutes, which caused the cells to burst, releasing their intracellular cAMP. After centrifugation, cAMP concentration was measured in the supernatants as described previously herein.

Statistical Analysis and Data Presentation

For statistical calculations and graphic presentations the maximum values of VV-induced histamine and leukotriene release were used. Analyses were performed using SPSS for Windows (SPSS Inc, Chicago, IL). To compare baseline values (intracellular cAMP, maximum histamine and leukotriene release, total histamine content) between patients and controls, the Mann-Whitney *U* test was used. The Wilcoxon test was used for comparisons within groups to determine differences before and during VIT. $P \leq .05$ was regarded as statistically significant. All the data are given as median and range.

RESULTS

Histamine Release

In patients treated with VIT for 1 week, maximum histamine release after stimulation with VV decreased significantly ($P = .04$), with the median changing from 21.6% before rush VIT to 11.2% after rush VIT (Fig 1). From baseline to the second evaluation after 1 week, the total histamine content of basophils (median, 20.4 vs 16.1 ng/mL; $P = .2$) and anti-

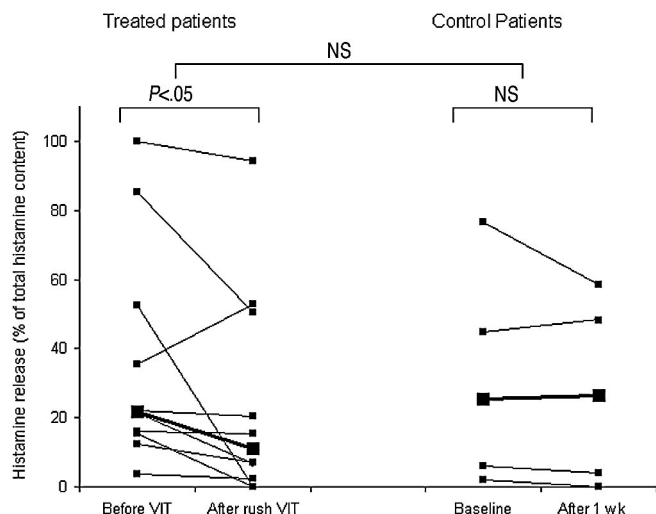


Figure 1. Maximum vespind venom-induced histamine release from basophils of vespind venom-allergic patients with ($n = 10$) or without ($n = 4$) rush venom immunotherapy (VIT). Medians are given in bold. NS indicates not significant.

IgE-induced histamine release (median, 16.5% vs 19.4%; $P = .6$) did not change significantly. In the 5 patients reevaluated after 6 months of VIT, decreased histamine release was no longer demonstrable. Median histamine release was 22.1% before VIT and 2.2% after rush VIT but was increased to 43.3% after 6 months of VIT ($P = .5$ compared with baseline). Baseline histamine release in controls ($n = 4$) did not differ significantly from that in VIT patients (25.3% vs 21.6%; $P = .57$). From baseline to the second evaluation after 1 week, VV-induced histamine release (25.3% vs 26.3%; $P = .47$) and total histamine content (37.8 vs 31.2 ng/mL; $P = .47$) of basophils did not change in controls.

Leukotriene Release

In patients treated with VIT for 1 week, maximum VV-induced sulfidoleukotriene production decreased significantly ($P = .01$), with the median changing from 1,184.0 pg/mL before rush VIT to 491.5 pg/mL after rush VIT (Fig 2). In the 5 patients reevaluated after 6 months of VIT, decreased sulfidoleukotriene release from basophils was no longer found. Median sulfidoleukotriene production changed from 1,682.0 pg/mL before VIT to 845.0 pg/mL after rush VIT and was again increased to 3,301 pg/mL after 6 months of VIT ($P = .2$ compared with baseline). Median baseline sulfidoleukotriene production in controls ($n = 5$) did not differ significantly from that in patients (4,483.0 vs 1,184.0 pg/mL; $P = .8$). Within 1 week, VV-induced sulfidoleukotriene production of basophils did not change significantly in controls (4,483.0 vs 1,698.0 pg/mL; $P = .5$).

Plasma cAMP

In the 10 patients treated with VIT, the median plasma cAMP concentration was 2.70 pmol before rush VIT and 3.67 pmol

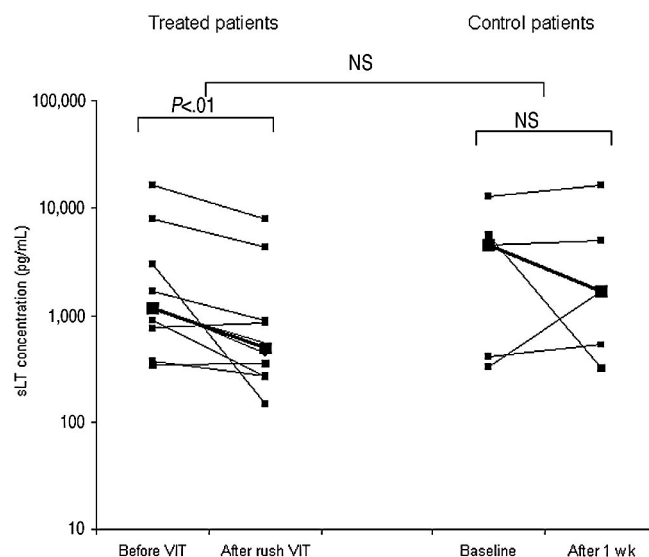


Figure 2. Maximum vespind venom-induced sulfidoleukotriene (sLT) production in basophils of vespind venom-allergic patients with ($n = 10$) or without ($n = 5$) rush venom immunotherapy (VIT). Medians are given in bold. NS indicates not significant.

after ($P = .45$). In the 5 patients reevaluated after 6 months of VIT the median plasma cAMP concentrations before, immediately after, and 6 months after rush VIT were 2.63, 3.61, and 2.43 pmol, respectively ($P = .89$ compared with pre-VIT values). In the control group there were no significant changes of the plasma cAMP levels in 1 week (3.03 vs 2.61 pmol; $P = .69$).

Intracellular cAMP Content

Compared with baseline values, median intracellular cAMP levels in PBLs were significantly increased after rush VIT (1.09 vs 2.03 pmol; $P = .047$) (Fig 3). In the 5 patients reevaluated after 6 months of VIT the increased intracellular cAMP level had returned to baseline: The medians before, immediately after, and 6 months after rush VIT were 1.08, 2.39, and 1.3 pmol, respectively ($P = .23$ compared with pre-VIT values) (Fig 4). The baseline intracellular cAMP content in controls ($n = 5$) did not differ significantly from that in patients (median, 1.83 vs 1.09 pmol; $P = .2$). Within 1 week, intracellular cAMP content did not change in controls (median, 1.83 vs 2.14 pmol; $P = .89$).

DISCUSSION

Immediately after rush vespind VIT, allergen-induced release of histamine and sulfidoleukotrienes from basophils was decreased, whereas intracellular cAMP levels in PBLs were increased in patients with VV allergy. Decreased histamine and sulfidoleukotriene release from basophils after reaching the maintenance dose by either rush or ultrarush VIT has already been reported.⁴⁻⁶ In contrast to other studies,^{4,6} there was neither a significant change in total histamine content of

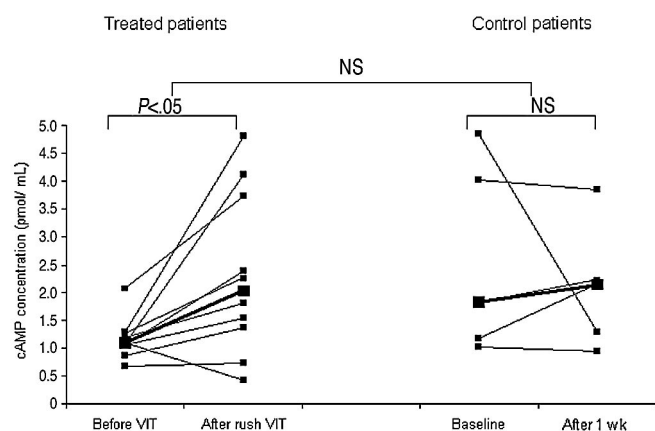


Figure 3. Intracellular cyclic adenosine monophosphate (cAMP) levels in peripheral blood leukocytes from vespid venom-allergic patients with ($n = 10$) or without ($n = 5$) rush venom immunotherapy (VIT). Medians are given in bold. NS indicates not significant.

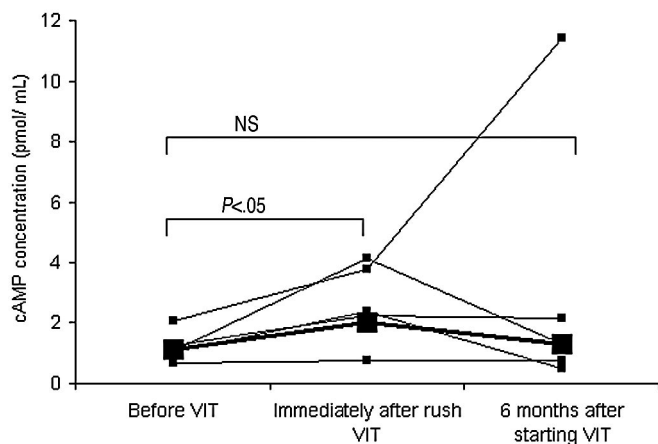


Figure 4. Intracellular cyclic adenosine monophosphate (cAMP) levels of peripheral blood leukocytes from 5 patients evaluated before, immediately after, and 6 months after rush venom immunotherapy (VIT). Medians are given in bold. NS indicates not significant.

basophils nor a decline in anti-IgE-induced histamine release immediately after the dose increase phase. However, we did not assess quantitatively the action of anti-IgE by using various concentrations of this stimulus, which might explain the differing results in part.

Decreased mediator release after rush VIT could be the reason why clinical protection is achieved immediately after reaching the maintenance dose of an ultrarush protocol.³ Loss of stored mediators,¹² "exhaustion" of mediator release because of permanent antigen stimulation,¹³ and increased production of immunoregulating IL-10 by T cells⁵ could be causes of decreased cell reactivity to allergen. However, decreased mediator release on allergen stimulation was found only at an early stage of VIT. Other mechanisms, eg, a switch

from a T_H2 to a T_H1 cytokine pattern or increasing IgG4 and decreasing IgE levels, may be responsible for clinical protection in the later course of treatment. Probably particularly the regulatory cytokine IL-10 then plays a crucial role.⁷

Up to now it has not been known by which mechanism allergen-induced mediator release is decreased early in VIT. In this study, increased intracellular cAMP levels in PBLs were associated with decreased releasability of basophils on stimulation with relevant allergen. Many cells contain the second messenger cAMP, and there is evidence that elevated cAMP levels in basophils reduce mediator release.^{8,9} Cyclic adenosine monophosphate is produced by an adenylate cyclase, which is a G-protein-dependent enzyme. G-proteins can be activated by many stimuli, eg, via β -adrenoreceptors by adrenaline or noradrenaline and also via histamine receptor types 1 (HR1) and 2 (HR2).¹⁴ Histamine stimulation of HR2 on T_H2 cells results in cAMP formation and the inhibition of IL-4 and IL-13 secretion.¹⁴ As histamine release from basophils inhibits itself via HR2,¹⁵ the continuing histamine release of effector cells during rush VIT and the subsequently induced HR2 stimulation might explain the elevated cAMP levels.

It also can be speculated that elevated cAMP levels are related to decreased activity of cAMP-deactivating phosphodiesterase. The activity of phosphodiesterase seems to play an important role in atopic diseases, eg, elevated intracellular phosphodiesterase levels were found in immune cells (lymphocytes, granulocytes, and mixed leukocytes) from patients with atopic dermatitis^{16,17} or allergic bronchial asthma,^{18,19} and increased activity of phosphodiesterase in leukocytes of patients with seasonal allergic rhinoconjunctivitis during the pollen season was related to disease activity.²⁰ However, the effect of immunotherapy on phosphodiesterase activity remains to be evaluated.

It is tempting to speculate whether cAMP-inducing drugs might be used for treatment of allergic diseases. Already in 1968 Lichtenstein and Margolis²¹ demonstrated that drugs such as catecholamines and methylxanthines inhibit antigen-induced histamine release in mixed leukocyte preparations. Peachell et al⁸ demonstrated that drugs that increase cAMP levels in mast cells and basophils inhibit IgE-triggered mediator release. A corresponding approach is the inhibition of cAMP degradation by drugs blocking cAMP-specific phosphodiesterase 4. Compounds such as rolipram and roflumilast stabilize the activity of lymphocytes,²² macrophages,²³ neutrophils,²⁴ eosinophils,²⁵ and basophils.^{26,27}

This study demonstrates that an increase in intracellular cAMP levels in PBLs is induced by rush VIT. This may be of importance for the early clinical efficacy of VIT as elevated cAMP levels decrease mediator release from effector cells. However, like the decreased mediator release from basophils, increased intracellular cAMP levels were found only immediately after rush VIT but not after 6 months of continued therapy. Thus, an increase in intracellular cAMP content is not a hallmark of long-term VIT.

Although transient elevated cAMP levels probably are related to the initial mechanisms of VIT, their significance for the efficacy of VIT needs further clarification. In case an increase in intracellular cAMP turns out to be essential for successful VIT, drugs that increase intracellular cAMP, eg, by pharmacologic inhibition of phosphodiesterase, could be useful for enhancing the efficacy of immunotherapy.

REFERENCES

- European Academy of Allergology and Clinical Immunology. Position paper: immunotherapy with Hymenoptera venoms. *Allergy*. 1993;48(suppl 14):36–46.
- Ruëff F, Przybilla B, Müller U, Mosbech H. The sting challenge test in Hymenoptera venom allergy. *Allergy*. 1996;51:216–225.
- Van der Zwan JC, Flinterman J, Jankowski IG, Kerckhaert JAM. Hyposensitisation to wasp venom in 6 hours. *BMJ*. 1983; 287:1329–1331.
- Jutel M, Müller UR, Fricker M, Rihs S, Pichler WJ, Dahinden C. Influence of bee venom immunotherapy on degranulation and leukotriene generation in human blood basophils. *Clin Exp Allergy*. 1996;26:1112–1118.
- Pierkes M, Bellinghausen I, Hultsch T, Metz G, Knop J, Saloga J. Decreased release of histamine and sulfidoleukotrienes by human peripheral blood leukocytes after VV immunotherapy is partially due to induction of IL-10 and IFN- γ production of T-cells. *J Allergy Clin Immunol*. 1999;103:326–332.
- Stephan V, Kühn J, Urbanek R. Relevance of basophil histamine release changes during venom immunotherapy. *Allergy*. 1989; 44:453–459.
- Akdis CA, Blesken T, Akdis M, Wüthrich B, Blaser K. Role of IL-10 in specific immunotherapy. *J Clin Invest*. 1998;102: 98–106.
- Peachell PT, MacGlashan DW Jr, Lichtenstein LM, Schleimer RP. Regulation of human basophil and lung mast cell function by cyclic adenosine monophosphate. *J Immunol*. 1988;140: 571–579.
- Botana LM, MacGlashan DW. Differential effects of cAMP-elevating drugs on stimulus-induced cytosolic calcium changes in human basophils. *J Leukoc Biol*. 1994;55:798–804.
- Eberlein-König B, Ullmann S, Thomas P, Przybilla B. Tryptase and histamine release due to a sting challenge in bee venom allergic patients treated successfully or unsuccessfully with hyposensitization. *Clin Exp Allergy*. 1995;25:704–712.
- Siraganian RP. Automated histamine analysis for in vitro allergy testing, II: correlation of skin test results with in vitro whole blood histamine release in 82 patients. *J Allergy Clin Immunol*. 1977;59:214–222.
- Siegmund R, Vogelsang H, Machnik A, Herrmann D. Surface membrane antigen alteration on blood basophils in patients with hymenoptera venom allergy under immunotherapy. *J Allergy Clin Immunol*. 2000;106:1190–1195.
- MacGlashan DW Jr, Mogowski M, Lichtenstein LM. Studies of antigen-binding on human basophils, II: continued expression of antigen-specific IgE during antigen-induced desensitization. *J Immunol*. 1983;130:2237–2242.
- Jutel M, Watanabe T, Klunker S, et al. Histamine regulates T-cell and antibody responses by differential expression of H1 and H2 receptors. *Nature*. 2001;413:420–425.
- Lichtenstein LM, Gillespie E. Inhibition of histamine release by histamine is controlled by an H2 receptor. *Nature*. 1973;244: 287–288.
- Parker CW, Eisen AZ. Altered cyclic AMP metabolism in atopic eczema. *Clin Res*. 1979;20:418.
- Reed CE, Busse WW, Lee TP. Adrenergic mechanisms and the adenylyl cyclase system in atopic dermatitis. *J Invest Dermatol*. 1976;67:333–338.
- Gillespie E, Valentine MD, Lichtenstein LM. Cyclic AMP metabolism in asthma: studies with leucocytes and lymphocytes. *J Allergy Clin Immunol*. 1974;53:27–33.
- Parker CW, Smith JW. Alterations in cyclic adenosine monophosphate metabolism in human bronchial asthma. *J Clin Invest*. 1973;52:48–59.
- Haen E, Bleise U, Przybilla B. Some alterations of the leucocyte β_2 -adrenoreceptor/cAMP-system in patients with seasonal allergic rhinoconjunctivitis are related to disease activity. *Clin Exp Allergy*. 1997;27:787–795.
- Lichtenstein LM, Margolis S. Histamine release in vitro: inhibition by catecholamines and methylxanthines. *Science*. 1968; 161:902–903.
- Robicsek SA, Blanchard DK, Djeu JY, Krzanowski JJ, Szenti-vanyi A, Polson JB. Multiple high-affinity cyclic AMP phosphodiesterases in human T-lymphocytes. *Biochem Pharmacol*. 1991;42:869–877.
- Turner NC, Wood LJ, Burns FM, Guerey T, Souness JE. The effect of cyclic AMP and cyclic GMP phosphodiesterase inhibitors on the superoxid burst of guinea pig peritoneal macrophages. *Br J Pharmacol*. 1993;108:876–883.
- Nielson CP, Vestal RE, Sturm RJ, Haeslip R. Effects of selective phosphodiesterase inhibitors on the polymorphonuclear leukocyte respiratory burst. *J Allergy Clin Immunol*. 1990;86: 801–808.
- Dent G, Gienbycz MA, Rabe KF, Barnes PJ. Inhibition of eosinophils cyclic nucleotide PDE activity and opsonized zymosan-stimulated respiratory burst by “type IV”-selective PDE inhibitors. *Br J Pharmacol*. 1991;103:1339–1346.
- Peachell PT, Undem BJ, Schleimer RP, et al. Preliminary identification and role of phosphodiesterase isozymes in human basophils. *J Immunol*. 1992;148:2503–2510.
- Weston MC, Anderson N, Peachell PT. Effectors of phosphodiesterase inhibitors on human lung mast cell and basophils function. *Br J Pharmacol*. 1997;121:287–295.

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