

Elevated circulating adenosine level potentiates antigen-induced immediate bronchospasm and bronchoconstrictor mediator release in sensitized guinea pigs

Éva Huszár, PhD,^a Ildikó Horváth, MD, PhD,^a Erzsébet Barát, PhD,^a Irén Herjavec, MD, PhD,^a György Böszörményi-Nagy, MD, PhD,^a and Márk Kollai, MD, PhD^b
Budapest, Hungary

Background: Adenosine causes bronchoconstriction in asthmatic patients, and it is also accepted that adenosine influences histamine release from activated human mast cells and basophils *in vitro*.

Objective: In this study we tested the hypothesis that adenosine potentiates both the airway narrowing and the release of bronchoconstrictor mediators induced by ovalbumin challenge in sensitized guinea pigs.

Methods: After ovalbumin sensitization, 4 groups were studied: control group, adenosine group (ADO), ovalbumin group (OA), and adenosine plus ovalbumin group (ADO + OA). Changes in airway resistance were assessed from continuously recorded pulmonary insufflation pressure (PIP). The concentration of histamine, PGD₂, and thromboxane B₂ were determined from bronchoalveolar lavage fluids.

Results: Adenosine alone (6 mg/kg intravenously) did not influence baseline values of PIP and the mediator concentrations; however, ovalbumin (10 mg/kg intravenously) increased both the PIP and the levels of the measured mediators compared with the control and ADO groups. When ovalbumin challenge was preceded by adenosine administration, both PIP and mediator levels were significantly enhanced compared with values obtained after simple ovalbumin provocation (ADO + OA vs OA: $P < .05$).

Conclusion: These results suggest that adenosine potentiates the airway narrowing induced by ovalbumin challenge and that this effect may develop through facilitation of the release of bronchoconstrictor mediators during the immediate airway response. (*J Allergy Clin Immunol* 1998;102:687-91.)

Key words: Adenosine, antigen challenge, histamine, PGD₂, thromboxane B₂, prostaglandins, asthma, guinea pigs

It is known that patients with allergies and asthmatic patients, but not normal individuals, react to inhaled

Abbreviations used

BAL:	Bronchoalveolar lavage
cAMP:	Adenosine 3': 5'-cyclic monophosphate
HR:	Heart rate
IP ₃ :	Inositol triphosphate
MABP:	Mean arterial blood pressure
PIP:	Pulmonary insufflation pressure
TXB ₂ :	Thromboxane B ₂

adenosine with a sustained bronchoconstriction.¹ Adenosine is present in higher concentrations in the bronchoalveolar lavage (BAL) fluid obtained from asthmatic patients compared with that found in normal control subjects.² In asthmatic patients, allergen-induced bronchospasm is associated with the elevation of plasma adenosine level,^{3,4} and *in vitro* allergen provocation leads to an immediate release of adenosine from mast cells and from actively sensitized rat lungs.^{5,6} On the other hand, adenosine was also found to influence the antigen-induced release of histamine from isolated human mast cells and basophils in a dose- and time-dependent manner.⁷⁻⁹ These results suggest that adenosine may play a part in the early response to allergen in asthmatic patients both by acting directly on bronchial smooth muscle and by influencing mediator release from the inflammatory cells.

The effect of adenosine on bronchial smooth muscle is mediated by different adenoreceptor subtypes (A₁, A_{2a}, A_{2b}, and A₃), activating different second-messenger mechanisms. Activation of the A₁ receptor, and to some extent the A₃ receptor, induces adenylyl cyclase inhibition with subsequent decrease in adenosine 3':5'-cyclic monophosphate (cAMP) level. This intracellular signal induces contraction of the airway smooth muscle. In contrast, when adenosine activates the A_{2a} receptor, cAMP level increases and smooth muscle becomes relaxed.¹⁰⁻¹³ The A₃ and A_{2b} receptors have been suggested to mediate the facilitation of antigen-induced secretion of bronchoconstrictor mediators. The second-messenger pathway of this effect has not been defined precisely, but there are some indications that increased inositol triphosphate (IP₃) and Ca²⁺ levels may be involved.^{10,14-18}

From ^athe Department of Pathophysiology, National "Korányi" Institute for TB and Pulmonology, and ^bthe Second Department of Physiology, Semmelweis Medical University, Budapest.

Supported by the Hungarian National Scientific Research Funds (OTKA T016270, OTKA F0170 50, and ETT T-06 146/96).

Received for publication Sept 25, 1997; revised Apr 20, 1998; accepted for publication May 27, 1998.

Reprint requests: Éva Huszár, PhD, Department of Pathophysiology, National "Korányi" Institute for TB and Pulmonology, Budapest, Pihenő u. 1, 1529-Hungary.

Copyright © 1998 by Mosby, Inc.

0091-6749/98 \$5.00 + 0 1/1/91948

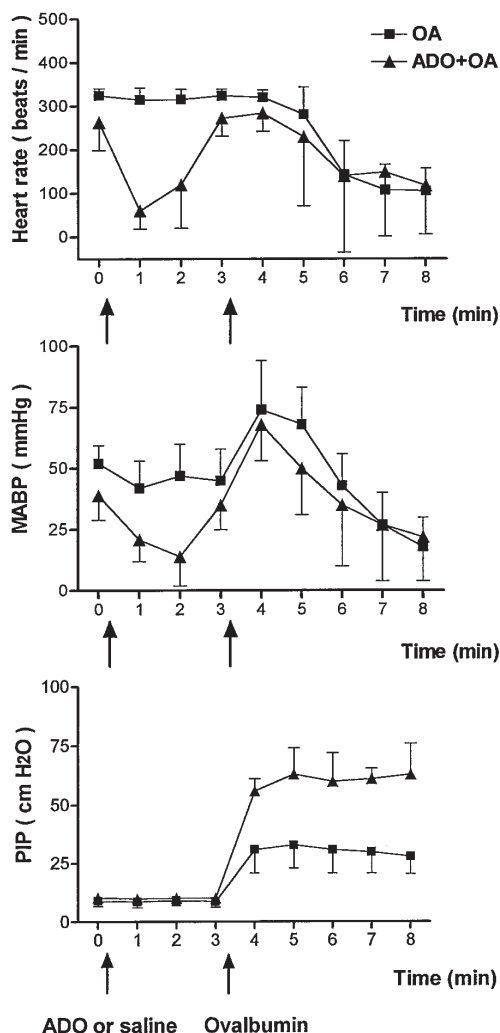


FIG 1. Time course for HR, MABP, and PIP responses to saline, then OA (OA group) and to ADO, then OA (ADO + OA group). OA, ADO + OA as in Table I.

The aim of this study was to test the hypothesis that intravenous administration of adenosine influences antigen-induced immediate bronchoconstriction and the release of bronchoconstrictor mediators. BAL fluid collection from asthmatic patients is limited by ethical considerations, therefore we performed our experiments on an established, well-characterized animal model for asthma, ovalbumin-sensitized guinea pigs. We measured both the pulmonary insufflation pressure (PIP) and the levels of histamine, PGD₂, and thromboxane B₂ (TXB₂) in BAL fluid obtained after ovalbumin or placebo challenge.

METHODS

Animals

Pathogen-free Dunkin-Hartley guinea pigs were used in this study (Lati Ltd, Budapest, Hungary). The animals were housed in individual cages in climate-controlled animal quarters and were given water and food ad libitum. For the care and use of animals, the guidelines of the National Research Council Guide were followed.

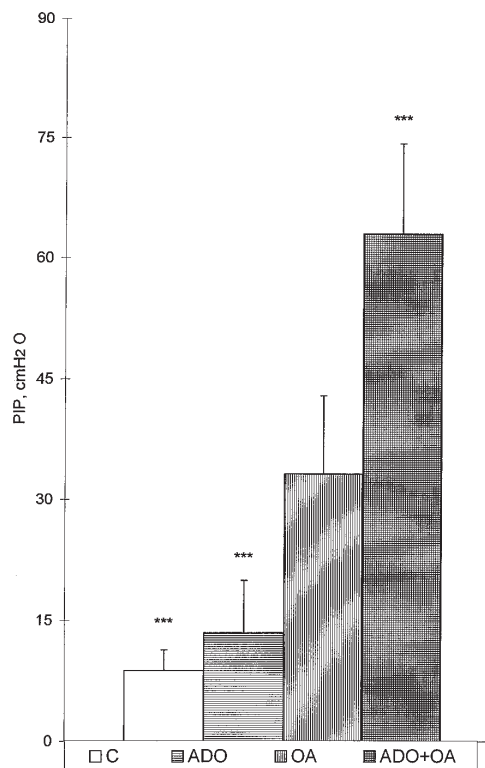


FIG 2. Peak values of PIP of guinea pigs in the different animal groups (mean $\pm$ 1 SD) *** $P < .001$ OA group vs control (C), ADO, and ADO + OA groups. Control, ADO, OA, and ADO + OA as in Table I.

Sensitization procedure

The guinea pigs (both sexes, weighing 350 to 450 g, $n = 27$) were sensitized to ovalbumin in two steps: the animals were injected intraperitoneally with 0.5 mL of saline containing 10 μ g ovalbumin (Sigma Chemical, St Louis, Mo) and 100 μ g Al(OH)₃ on day 1 and 10 mg/kg ovalbumin intraperitoneally on day 5.

Surgical procedure

Anaphylactic challenge was performed on days 21 to 27. Animals were anesthetized intraperitoneally with urethane (Hun-garopharma, Budapest) (2 g/kg). After cannulas were introduced into the trachea and the vein, immobilization was performed with pipecuronium bromate (Arduan, Chemical Works of Gedeon Richter Ltd, Budapest) (0.1 mg/kg intravenously). To maintain guinea pigs on artificial ventilation, we used a rodent ventilator (Harvard Apparatus, Dover, Mass) delivering a tidal volume of 3.5 mL room air at a frequency of 40 breaths per minute. After the surgical procedure was completed, 20 minutes was allowed for stabilization of cardiorespiratory parameters and then mean arterial blood pressure (MABP), heart rate (HR), and PIP were continuously recorded (Pulmodyn program package, version 1.511, 1989, Hugo Sachs Electronic, Germany). Values of MABP, HR, and PIP were averaged over a 1-minute period to reduce error caused by random and deterministic data variability. MABP and HR occasionally exhibited slow wave fluctuations over a period of 10 to 25 seconds. Data were averaged both at baseline conditions and after ovalbumin challenge as well.

TABLE I. Baseline cardiovascular and pulmonary parameters (mean $\pm$ 1 SD)

	HR (beats/min)	MABP (mm Hg)	PIP (cm H ₂ O)
Control	311 $\pm$ 29	45 $\pm$ 13	9 $\pm$ 2.5
ADO	291 $\pm$ 9	47 $\pm$ 13	13 $\pm$ 3.6
OA	324 $\pm$ 16	52 $\pm$ 7.5	9 $\pm$ 2.3
ADO + OA	263 $\pm$ 63	39 $\pm$ 10	11 $\pm$ 1.8

Control group, saline-treated and saline-challenged, $n = 7$; ADO group, adenosine-treated and saline-challenged, $n = 6$; OA group, saline-treated and ovalbumin-challenged, $n = 7$; ADO + OA group, adenosine-treated and ovalbumin-challenged, $n = 7$.

TABLE II. Concentrations of bronchoconstrictor mediators in BAL fluids (pg/mL⁻¹, mean $\pm$ 1 SD)

	Control	ADO	OA	ADO + OA
Histamine	450 ($\pm$ 362)	440 ($\pm$ 377)	1620 ($\pm$ 1884)	12,140 ($\pm$ 7085) [†]
PGD ₂	57 ($\pm$ 15.1) [‡]	53 ($\pm$ 11.5) [‡]	142 ($\pm$ 37)	438 ($\pm$ 190) [†]
TXB ₂	65 ($\pm$ 12.7) [*]	56 ($\pm$ 12.6) [*]	709 ($\pm$ 652)	1878 ($\pm$ 1016) [*]

OA group vs control, ADO, and ADO + OA groups: ^{*} $P < .05$, [†] $P < .01$, [‡] $P < .001$. Control, ADO, OA, and ADO + OA groups as in Table I.

Provocation

After baseline parameters were recorded, the animals were injected intravenously with the following agents: control group (C, $n = 7$): saline and after 3 minutes, saline again; adenosine group (ADO, $n = 6$): 6 mg/kg adenosine in saline and after 3 minutes, saline; ovalbumin group (OA, $n = 7$): saline and after 3 minutes, 10 mg/kg ovalbumin; adenosine plus ovalbumin group (ADO + OA, $n = 7$): 6 mg/kg adenosine and after 3 minutes, 10 mg/kg ovalbumin. Adenosine (Sigma Chemical, St Louis, Mo) and ovalbumin were prepared in saline. All parameters were measured for 5 minutes after saline (as placebo) or ovalbumin challenges.

Bronchoalveolar lavage

BAL was performed with saline (3×2.5 mL, 25°C) immediately after disconnection from the respiratory pump. Saline contained 10^{-5} mmol/mL indomethacin to avoid in vitro production of cyclooxygenase metabolites of arachidonic acid and 5×10^{-3} mmol/mL EDTA to inhibit metabolism of histamine by diamine oxidase.¹⁹ Lavage fluids were injected and recovered through a polyethylene tracheal cannula and were then transferred into a polypropylene tube precooled to 4°C. The volume of BAL fluids was measured and the total cell count was estimated in a Burkner chamber. To remove the cells the samples were centrifuged (Janet-zky, K26, 1000g, 4°C, 20 minutes), and the protein concentration of supernatants was determined by the method of Lowry.²⁰ The samples were divided into 3 parts and stored at -20°C until determination of metabolite concentration (maximum 4 weeks).

Histamine, PGD₂, and TXB₂ determination

All bronchoconstrictor metabolites were measured from the supernatant of BAL fluids by immunodiagnostic kits (histamine from Immunotech, Marseille, France, PGD₂ and TXB₂ from Institute of Isotopes Co, Ltd, Budapest). Detection limits of PGD₂ and TXB₂ were 12 pg/mL and 111 pg/mL of histamine, respectively.

Statistical analysis

Results are expressed as mean $\pm$ 1 SD. One-way ANOVA and post hoc t tests were used to test differences among groups. Differences were considered significant at $P < .05$. Correlation between the PIP values and measured metabolites were performed by linear regression analysis. Two-way analysis of variance (group $\times$ effect) with repeated measures was used to test for differences of cardiovascular and pulmonary parameters between OA and ADO + OA groups as a function of time.

RESULTS

There were no substantial differences in baseline MABP, HR, and PIP values among the different groups (Table I). Changes of cardiovascular and pulmonary parameters in response to saline or adenosine and ovalbumin in the OA and ADO + OA groups are shown in Fig 1. Immediately after adenosine treatment, baseline MABP and HR decreased and then returned to baseline values within 3 minutes; however, baseline PIP values did not change significantly with adenosine.

In the saline-pretreated group, ovalbumin induced an immediate and sustained increase in PIP accompanied by a moderate temporary increase in MABP, with no change in HR in the first minute. Both MABP and HR values decreased from the second minute after ovalbumin administration in this group (Fig 1). In the ADO + OA group, adenosine pretreatment did not significantly influence the effect of ovalbumin on MABP and HR but facilitated the ovalbumin-induced increase in PIP (Fig 1).

Peak PIP values in the different groups are shown in Fig 2. These values were obtained in the following way. PIP values were averaged over 1-minute periods for 5 minutes after OA challenge, and out of the 5 values the highest was chosen. There was no difference in peak PIP values between the control group and the adenosine groups (control vs ADO: 8.8 ± 2.5 vs 13.5 ± 6.5 cm H₂O, $P > .05$). In the ovalbumin-provoked group, peak PIP values were significantly higher compared both with the control and the ADO groups (OA: 33.2 ± 9.7 cm H₂O, $P < .001$ for control and ADO vs OA). Adenosine pretreatment enhanced the ovalbumin-induced bronchoconstriction as it was reflected by increased peak PIP values (ADO + OA: 63.0 ± 11.4 cm H₂O, $P < .001$ for OA vs ADO + OA).

Concentrations of histamine, PGD₂, and TXB₂ in BAL fluids

There were no differences in the volumes and protein concentrations of recovered BAL fluids in the different groups. The mean ($\pm$ 1 SD) concentrations of histamine,

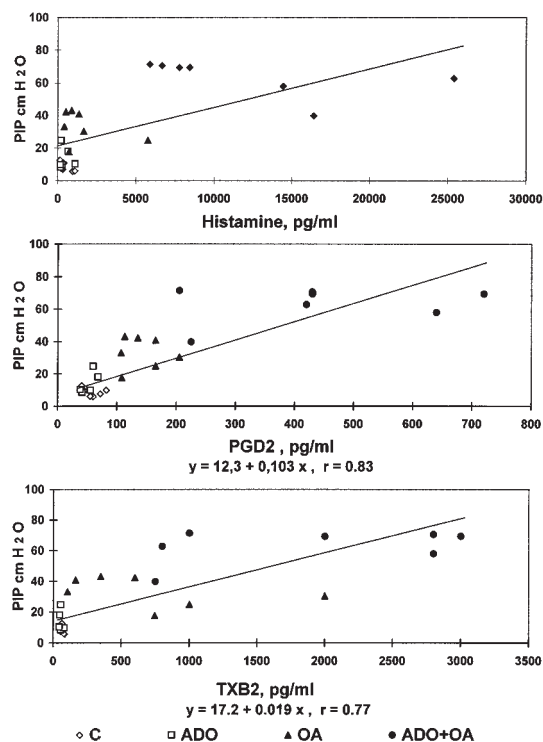


FIG 3. Regression of actual peak PIP responses against concentrations of histamine, PGD₂, and TXB₂ in BAL fluids ($n = 27$). ADO, OA, ADO + OA, as in Table I.

PGD₂ and TXB₂ in BAL fluids are given for the different groups in Table II. The concentrations of bronchoconstrictor metabolites did not differ in the control and ADO groups. After ovalbumin provocation, the concentration of all 3 metabolites increased, although the change in histamine concentration was not significant. The concentration of the measured metabolites further increased in the ADO + OA group (significance levels between OA vs control, ADO, and ADO + OA groups are shown in Table II).

Correlation analyses between changes of PIP values and concentrations of metabolites

When data from the control, ADO, OA, and ADO + OA groups were pooled, a significant linear relation was found between changes of PIP values and corresponding levels of bronchoconstrictor metabolites (Fig 3). PIP values were related most closely to PGD₂ concentrations ($r^2 = 0.83$).

DISCUSSION

Histamine was shown to be the primary mediator involved in the immediate airway response, but cyclooxygenase mediators of arachidonic acid were also found to play a role in the development of the early asthmatic attack.^{21,22} PGD₂ and TXA₂ both have bronchoconstrictor effects on airway smooth muscle, with PGD₂ being 30 times more potent than histamine.²³ For this reason we investigated the effect of intravenous adenosine not only on antigen-induced bronchospasm

but also on the levels of the above-mentioned bronchoconstrictor mediators as well.

The possible direct effect of intravenous adenosine (0.03 to 6.0 mg/kg, $n = 6$) on PIP values was examined in preliminary experiments. Adenosine did not produce significant effects on baseline PIP values at any concentration used. Therefore adenosine in a dose of 6 mg/kg was administered to the animals both in ADO and ADO + OA groups to exert maximal effects because this dose represents the upper limit of adenosine solubility in aqueous solutions.

Adenosine by itself did not influence bronchial tone and levels of BAL mediators. However, when the guinea pigs were challenged with ovalbumin after adenosine administration (ADO + OA group), both the antigen-induced airway narrowing and the release of the preformed histamine and production of the newly formed PGD₂ and TXA₂ were enhanced compared with the OA group. These observations are consistent with the results of others. Larsson and Sollevi²⁴ found that adenosine infusion did not change bronchial tone in asthmatic patients. Similarly, no changes in basal airway resistance were found by Breschi et al²⁵ after intravenous injection of 0.03 to 3.0 mg/kg adenosine in guinea pigs, but adenosine could significantly enhance the bronchocontractile response to histamine.

Adenosine may exert its potentiating effect by increasing second-messenger levels to subthreshold concentrations, which then would result in an enhanced response to subsequent ovalbumin administration. This effect of adenosine may be mediated by A₃ receptors, A_{2b} receptors, or both. Ramkumar et al^{14,18} and Marquardt et al¹⁷ suggested that adenosine or specific adenosine analogs to A₃ or A_{2b} receptors might activate phospholipase C. Increased enzyme activation may enhance IP₃ and Ca²⁺ levels, and elevated Ca²⁺ levels could subsequently facilitate the antigen-induced secretion of bronchoconstrictor mediators.

The correlation found between PIP values and the measured bronchoconstrictor mediator levels also support the hypothesis that the potentiating effect of adenosine on ovalbumin-induced bronchoconstriction might involve the enhanced histamine release and PGD₂ thromboxane formation in the airway wall. The fact that PIP values were related least closely to histamine may be attributed to the relatively higher variability of histamine concentration than that of PGD₂ and TXB₂. Other investigators have also found that BAL histamine levels of guinea pigs (nonsensitized, nonchallenged, and OA-sensitized, OA-challenged guinea pigs as well) show high variability.^{26,27} In addition, the time period at which release of histamine becomes full in response to allergen is species dependent. In BAL white cells obtained from monkeys, the complete release time was approximately 10 minutes. This is slower than the release of histamine from rat mast cells, which is complete in 1 minute and the release from isolated human lung mast cells, which is complete within 2 minutes.²⁸ A study investigating the time course of bronchoconstrictor mediator release in

isolated, perfused lung from sensitized guinea pigs showed that histamine, but not thromboxane, release was complete within 10 minutes.²⁹ Nambu et al³⁰ found that BAL histamine level was maximal at 1 minute after OA challenge (0.5 mg/kg intravenously) and was still elevated at 5 minutes after the challenge in sensitized guinea pigs *in vivo*. In the same set of experiments, TXB₂ level reached its maximal concentration at 5 minutes after provocation. In this study, a higher dose of ovalbumin (10 mg/kg) was used for challenge. Difference in the dose of applied ovalbumin may cause a difference in the time course of mediator release; however, we could not find any further information about this question. Therefore we relied on Nambu's data and performed lavages 5 minutes after challenges, assuming that histamine release is still activated and prostaglandin synthesis is already increased at this time point. As our results show, both the ovalbumin-induced histamine release and formation of cyclooxygenase products were enhanced by adenosine at this time point (ADO + OA vs OA). Only the levels of TXB₂ and PGD₂, but not the concentration of histamine, were significantly elevated in the saline-treated, ovalbumin-challenged group compared with the control group (OA vs control). Although presumably histamine release was not maximal at the chosen time point, the proof that mast cells were still activated at 5 minutes after ovalbumin provocation is the increased level of PGD₂, which is almost exclusively derived from mast cells, and enhanced histamine release caused by adenosine in ovalbumin-challenged (ADO + OA vs OA) but not saline-challenged guinea pigs (ADO vs control).

In summary, our study demonstrates that elevation in circulating adenosine level causes enhanced bronchospasm and facilitated release of preformed and newly formed bronchoconstrictor mediators induced by antigen challenge. These results suggest that inhibition of adenosine receptors on the inflammatory cells and airway smooth muscle cells may be useful in the treatment of patients with asthma.

REFERENCES

- Cushley MJ, Tattersfield AE, Holgate ST. Inhaled adenosine and guanosine on airway resistance in normal and asthmatic subjects. *Br J Clin Pharmacol* 1983;15:161-5.
- Driver AG, Kukoly CA, Ali S, Mustafa SJ. Adenosine in bronchoalveolar lavage fluid in asthma. *Am Rev Respir Dis* 1993;148:91-7.
- Holgate ST, Cushley MJ, Mann JS, Hughes P, Church MK. The action of purines on human airways. *Arch Int Pharmacodyn* 1986;280:240-52.
- Mann JS, Holgate ST, Renwick AG, Cushley MJ. Airway effects of purine nucleosides and nucleotides and release with bronchial provocation in asthma. *J Appl Physiol* 1986;61:1667-76.
- Fredholm BB. Release of adenosine from rat lung by antigen and compound 48/80. *Acta Physiol Scand* 1981;111:507-8.
- Marquardt DL, Gruber HE, Wasserman SI. Adenosine release from stimulated mast cells. *Proc Natl Acad Sci U S A* 1984;81:192-6.
- Hughes PJ, Holgate ST, Church MK. Adenosine inhibits and potentiates IgE-dependent histamine release from human lung mast cells by an A₂-purino-receptor mediated mechanism. *Biochem Pharmacol* 1984;33/23:3847-52.
- Church MK, Holgate ST, Hughes PJ. Adenosine inhibits and potentiates IgE-dependent histamine release from human lung basophils by an A₂-purinoreceptor mediated mechanism. *Br J Pharmacol* 1983;80:719-26.
- Peters ST, Schulman ES, Schleimer RP, Macglashan DW, Newball HH, Lichtenstein LM. Dispersed human lung mast cells: pharmacologic aspects and comparison with human lung tissue fragments. *Am Rev Respir Dis* 1982;126:1034-9.
- Olah ME, Stiles GL. Adenosine receptor subtypes: characterization and therapeutic regulation. *Ann Rev Pharmacol Toxicol* 1995;15:581-606.
- Ali S, Mustafa SJ, Metzger WJ. Adenosine-induced bronchoconstriction and contraction of airway smooth muscle from allergic rabbits with late-phase airway obstruction: evidence for an inducible adenosine A₁ receptor. *J Pharmacol Exp Ther* 1994;268/3:1328-34.
- Finney MJB, Karlsson JA, Persson CGA. Effects of bronchoconstrictors and bronchodilators on a novel human small airway preparation. *Br J Pharmacol* 1985;85:29-36.
- Farmer SG, Canning BJ, Wilkins DE. Adenosine receptor mediated contraction and relaxation of guinea pig isolated smooth muscle: effect of adenosine antagonists. *Br J Pharmacol* 1988;95:371-8.
- Ramkumar V, Stiles GL, Beaven MA, Ali H. The A₃ adenosine receptor is the unique adenosine receptor which facilitates release of allergic mediators in mast cells. *J Biol Chem* 1993;268/23:16887-90.
- Björk T, Gustafsson LE, Dahlén SE. Isolated bronchi from asthmatics are hyperresponsive to adenosine, which apparently acts indirectly by liberation of leukotrienes and histamine. *Am Rev Respir Dis* 1992;145:1087-91.
- Van Schaick EA, Jacobson KA, Kim HO, Jzerman AP, Danhof M. Hemodynamic effects and histamine release elicited by the selective adenosine A₃ receptor agonist 2-CI-IB-MECA in conscious rats. *Eur J Pharmacol* 1996;308:311-4.
- Marquardt DL, Walker LL, Heinemann S. Cloning of two adenosine receptor subtypes from mouse bone marrow-derived mast cells. *J Immunol* 1994;152:4508-15.
- Ramkumar V, Wilson M, Dhandaj DN, Gettys TW, Ali H. Dexamethasone upregulates A₃ adenosine receptors in rat basophilic leukemia (RBL-2H3) cells. *J Immunol* 1995;154:5436-43.
- Cushley MJ, Holgate ST. Adenosine-induced bronchoconstriction in asthma: role of mast cell-mediator release. *J Allergy Clin Immunol* 1985;75:272-8.
- Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951;193:265-8.
- 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 airway allergic asthmatics. *Am Rev Respir Dis* 1991;144:51-8.
- Crimi N, Palermo F, Polosa R, Oliveri R, Maccarone C, Palermo B, et al. Effect of indomethacin on adenosine-induced bronchoconstriction. *J Allergy Clin Immunol* 1989;83:921-5.
- Wenzel SE, Westcott JY, Smith HR, Larsen GL. Spectrum of prostanoid release after bronchoalveolar allergen challenge in atopic asthmatic and in control groups. *Am Rev Respir Dis* 1989;139:450-7.
- Larsson K, Sollevi A. Influence of infused adenosine on bronchial tone and bronchial reactivity in asthma. *Chest* 1988;93:280-4.
- Breschi MC, Nier P, Lazzeri N, Martinotti E. Adenosine enhances the bronchocontractile response to histamine in anaesthetized and curarized guinea pigs through a mechanism partly blocked by hexamethonium. *Pharmacology* 1994;49:42-51.
- Lawrence BP, Ingenito EP, Ingram RH Jr. Baseline lung function and inflammatory indices in guinea pigs: effects of growth, repeated lavage, and orotracheal intubation. *Exp Lung Res* 1990;16:617-26.
- Ingenito EP, Pliss LB, Martins MA, Ingram RH Jr. Effects of capsaicin on mechanical, cellular, and mediator response to antigen in sensitized guinea pigs. *Am Rev Respir Dis* 1991;143:572-7.
- Butchers PR, Vardey IF, Skidmore A, Wheelton A, Boutal LE. Histamine-containing cells from bronchial lavage of macaque monkeys: time course and inhibition of anaphylactic histamine release. *Int Arch Allergy Appl Immunol* 1980;62:205-12.
- Turner NC, Dollery CT. Release of arachidonic acid metabolites and histamine from sensitized guinea-pig lung following antigen challenge. *Br J Pharmacol* 1988;93:751-8.
- Nambu F, Murakata S, Shirai T, Omawari N, Sawada M, Okegawa T, et al. OKY-046 inhibits anaphylactic bronchoconstriction and reduces histamine level in bronchoalveolar lavage fluid in sensitized guinea pigs. *Prostaglandins* 1990;39:623-37.