

Role of cyclic 3',5'-adenosine monophosphate in the regulation of chemical mediator release and cytokine production from cultured human mast cells

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Background: Cultured human mast cells are known to resemble human lung mast cells in terms of the profiles of intracellular protease, the characteristics of histamine release, and the pharmacologic properties.

Objective: The role of cyclic 3',5'-adenosine monophosphate (cAMP) in chemical mediator release and cytokine production by human mast cells was determined.

Methods: We investigated the effects of cAMP-elevating agents on IgE-mediated chemical mediator release and cytokine production by cultured human mast cells. We also examined the relationship between intracellular cAMP levels and the inhibition of chemical mediator release or cytokine production by various drugs.

Results: β -agonists significantly suppressed IgE-mediated release of histamine, leukotrienes, and PGD₂ (chemical mediators) and the production of GM-CSF, IL-5 and macrophage inflammatory protein-1 α (cytokines). Phosphodiesterase inhibitors (theophylline, rolipram, and cilostazol) had no effect on chemical mediators but suppressed cytokine production.

Dibutyryl cAMP significantly suppressed both chemical mediator release and cytokine production, suggesting that their induction was regulated by intracellular cAMP. Elevation of cAMP by β -agonists at 10 minutes after treatment correlated well with the inhibition of histamine release. There was a significant relationship between cAMP elevation at 180 minutes and the inhibition of GM-CSF production at 360 minutes by β -agonists, rolipram, or cilostazol. Although 100 μ M/L theophylline significantly inhibited GM-CSF production, it had no effect on cAMP. **Conclusion:** Elevation of cAMP may be responsible for the inhibitory effect of β -agonists, rolipram, and cilostazol on chemical mediator release and cytokine production by cultured human mast cells. In contrast, theophylline may inhibit GM-CSF production independently of cAMP. (*J Allergy Clin Immunol* 1999;103:S421-8.)

Key words: Mast cell, histamine, leukotrienes

Recently, cultured human mast cells have been made available by culturing cord blood mononuclear cells for more than 10 weeks in the presence of stem-cell factor

Abbreviations used

cAMP: Cyclic 3',5'-adenosine monophosphate
LTs: Sulfido-leukotrienes
MIP: Macrophage inflammatory protein
SCF: Stem-cell factor

(SCF), IL-6, and PGE₂.^{1,2} The characteristics of these cultured mast cells have been examined by some investigators. Saito et al^{1,2} demonstrated that the cells contained about 3.6 μ g of histamine and 3.5 μ g tryptase per 10⁶ cells and expressed IgE high-affinity receptor (Fc ϵ RI) on their surface. These cultured mast cells released preformed mediator (histamine),² newly generated arachidonic acid metabolites (sulfido-leukotrienes [LTs] and PGD₂),^{2,3} and newly generated cytokines (TNF- α , GM-CSF, IL-5, and macrophage inflammatory protein-1 α (MIP-1 α)) on stimulation through cell surface IgE.^{2,4} More than 99% of the cultured mast cells contained tryptase-positive granules, and 20% to 30% had chymase-positive granules.^{1,2,5,6} Therefore these cultured cells are mainly tryptase-positive mast cells⁷ like those seen in human lung tissue and the gastrointestinal mucosa. Igarashi et al⁶ suggested that these cells resemble human lung mast cells in terms of their histamine release, because histamine is released by stimulation with anti-IgE or calcium ionophore A23187 but not by stimulation with compound 48/80 or substance P. Our recent studies have also indicated that the pharmacologic properties of cultured mast cells resemble those of human lung mast cells.³ Therefore cultured mast cells may be useful for biologic and pharmacologic studies of human lung mast cells.

Both β -adrenoceptor agonists (β -agonists) and phosphodiesterase inhibitors have been widely used for the treatment of asthma as bronchodilators. It is also well known that β -agonists are potent inhibitors of mediator release from human mast cells. β -agonists inhibit antigen-induced release of chemical mediators (such as histamine, LTs, and PGD₂) from human lung tissue fragments⁸⁻¹² and from isolated human lung mast cells.¹³⁻¹⁵ In contrast to β -agonists, there have been few studies examining the effects of phosphodiesterase inhibitors on the release of mediators from human mast cells. In our previous study,³ rolipram (100 μ M/L) weakly but significantly inhibited the release of histamine, LTs, and

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PGD₂, whereas other phosphodiesterase inhibitors (theophylline and cilostazol) had no effect. Therefore the effects of phosphodiesterase inhibitors on mediator release from human mast cells remain unclear.

β -agonists are known to activate adenylate cyclase and then to elevate intracellular cyclic 3',5'-adenosine monophosphate (cAMP) levels, although phosphodiesterase inhibitors also elevate cAMP levels. Peachell et al¹⁴ found that the elevation of cAMP was correlated quite well with the inhibition of IgE-mediated release of histamine and LTC₄ from human lung mast cells.

In the present study, to determine the role of cAMP in the release of chemical mediators and the production of cytokines by human mast cells, we examined the effects of β -agonists (isoproterenol and salbutamol) and phosphodiesterase inhibitors (theophylline, rolipram, and cilostazol) on the IgE-mediated release of histamine, LTs, and PGD₂ and the production of GM-CSF, IL-5, and MIP-1 α by cultured human mast cells. We also examined the relationship between the elevation of cAMP levels in mast cells and the inhibition of mediator release or cytokine production by these cAMP-elevating agents.

METHODS

Reagents

Isoproterenol, salbutamol, and theophylline (Sigma Chemicals, St Louis, Mo), rolipram and cilostazol (Nissan Chem Ind Ltd, Tokyo, Japan), and dibutyl cAMP (Wako Pure Chem Ind Ltd, Osaka, Japan) were dissolved in dimethyl sulfoxide. In preliminary experiments, the concentrations of dimethyl sulfoxide in working dilutions used in the present study (<0.1%) had no effect on the release of histamine, LTs, and PGD₂ or on the production of GM-CSF, IL-5, and MIP-1 α by cultured mast cells.

Mast-cell culture

Cultured mast cells were obtained from human umbilical cord blood cells by the method described previously with some modifications.^{1,2} Briefly, mononuclear cells were separated from heparinized umbilical cord blood on a Ficoll (Organon Teknica Co, Durham, NC) gradient. CD34⁺ cells were purified from the mononuclear cells with a CD34⁺ progenitor cell isolation kit (Miltenyi Biotec, Bergisch Gladbach, Germany) and a magnetic cell sorting system (Miltenyi Biotec). Purified CD34⁺ cells were cultured in α -minimum essential medium (GIBCO-BRL Laboratories, Grand Island, NY) containing 15% FBS (Filtron Pty Ltd, Brooklyn, Australia) in the presence of 80 ng/mL human recombinant SCF (Kirin Brewery, Maebashi, Japan) and 50 ng/mL human recombinant IL-6 (Kirin). Cells were harvested weekly and resuspended in the fresh medium. The purity of the mast cells was determined by toluidine blue and May-Gruenwald-Giemsa staining. Immunoperoxidase staining for tryptase and chymase was performed by previous method.¹⁶ Cultured mast cells used in the present study were 92.0 $\pm$ 2.9 days old ($n = 35$). Cells were almost 100% positive for tryptase and about 14% positive for chymase on immunophenotypic analysis.

Chemical mediator release

Mast cells were sensitized with human myeloma IgE (Chemicon Int Inc, Temecula, Calif) at 37°C overnight. In the experiment on IgE-mediated chemical mediator release (histamine, LTs, and PGD₂), cells were resuspended at 1 $\times$ 10⁵ cells/mL in Tyrode's solution containing 1 mmol/L CaCl₂, 0.6 mmol/L MgCl₂, 0.03% BSA

(Seikagaku Co, Tokyo, Japan) at pH 7.4 and were treated with several cAMP-elevating agents for 10 minutes and then were challenged by anti-human IgE (Cooper Biomedical, Inc, Malvern, Pa) at 4 μ g/mL for 30 minutes. In our preliminary experiments, the chemical mediator release increased in a dose-dependent manner (eg, anti-IgE-induced histamine release; anti-IgE at 40 ng/mL, 1.4% $\pm$ 0.6%; 400 ng/mL, 3.7% $\pm$ 2.3%; 4 μ g/mL, 10.8% $\pm$ 3.9%; 20 μ g/mL, 21.3% $\pm$ 0.4%; $n = 3$). We wanted to use the near optimum concentration of anti-IgE for the pharmacologic studies, but the concentration for optimal chemical mediator release was so high that it could have decreased the effect of the drugs. Therefore we used anti-IgE at 4 μ g/mL for chemical mediator assays.

Cytokine production

To examine the effect of the agents on cytokine production (GM-CSF, IL-5, and MIP-1 α), cells were resuspended at 5 $\times$ 10⁵ cells/mL in fresh culture medium and were treated with agents for 10 minutes and then were challenged with anti-human IgE (Cooper Biomedical, Inc, Malvern, Pa) at 2 μ g/mL for 6 hours. In previous studies, Saito et al² showed that histamine release 30 minutes after anti-IgE challenge increased dose-dependently whereas TNF- α production 360 minutes after challenge was maximal at 1.5 μ g/mL of anti-IgE. Ebisawa et al⁴ showed that the dose-response of GM-CSF, IL-5, and MIP-1 α was similar to that of TNF- α . We confirmed that production of these cytokines peaked at approximately 2 μ g/mL of anti-IgE. The reason the optimum concentration of anti-IgE differs for chemical mediator release and cytokine production is not clear. Anti-IgE (4 μ g/mL)-induced chemical mediator release reached a plateau within 30 minutes, whereas anti-IgE (2 μ g/mL)-induced cytokine production reached a plateau at about 360 minutes after challenge.

Measurement of chemical mediators and cytokines

The concentration of histamine released into the supernatant was quantified by postcolumn derivatization, as reported previously.¹⁷ For the analysis of LTs, we used a commercial ELISA kit (Bühlmann Laboratory, Allschwil, Switzerland), which is based on an mAb that recognizes LTs (LTC₄, LTD₄, and LTE₄) equally.¹⁸ The PGD₂ concentration was measured by a PGD₂-MOX EIA kit (Cayman Chemical Co, Ann Arbor, Mich). The kit was based on the conversion of PGD₂ to a stable methoxime derivative by treatment with methoxime hydrochloride.¹⁹ The concentrations of GM-CSF, IL-5, and MIP-1 α were measured by ELISA kits (R&D Systems, Inc, Minneapolis, Minn). The minimum detectable concentration of histamine, LTs, PGD₂, GM-CSF, IL-5, and MIP-1 α was 200, 41, 3.2, 1.5, 7.8, and 31.3 pg/mL, respectively. Measurements were performed in duplicate. Histamine release was expressed as a percentage of the total histamine content determined by cell lysis with perchloric acid. The inhibitory effects of drugs on release of each mediator were expressed as the percent inhibition relative to control release. Although cultured mast cells showed marked variability in anti-IgE-induced mediator release and cytokine production (histamine, 7.34% to 27.29% of the total histamine content; LTs, 2.1 to 8.2 ng/10⁶ cells; PGD₂, 3.1 to 12.2 ng/10⁶ cells; GM-CSF, 0.1 to 1.9 ng/10⁶ cells; IL-5, 0.1 to 1.1 ng/10⁶ cells; MIP-1 α , 0.7 to 6.5 ng/10⁶ cells), the inhibitory effects of the drugs were uniform.

Preparation of samples for cAMP analysis

The cAMP levels were measured according to the method of Vergheze et al.²⁰ Cells were resuspended at 5 $\times$ 10⁵ cells/mL in Tyrode's solution or culture medium and were incubated with cAMP-elevating agents in the presence of anti-IgE in a total volume of 100 μ L. The reaction was terminated by snap freezing in liquid nitrogen. After the thawing and acetylating of samples, a cAMP

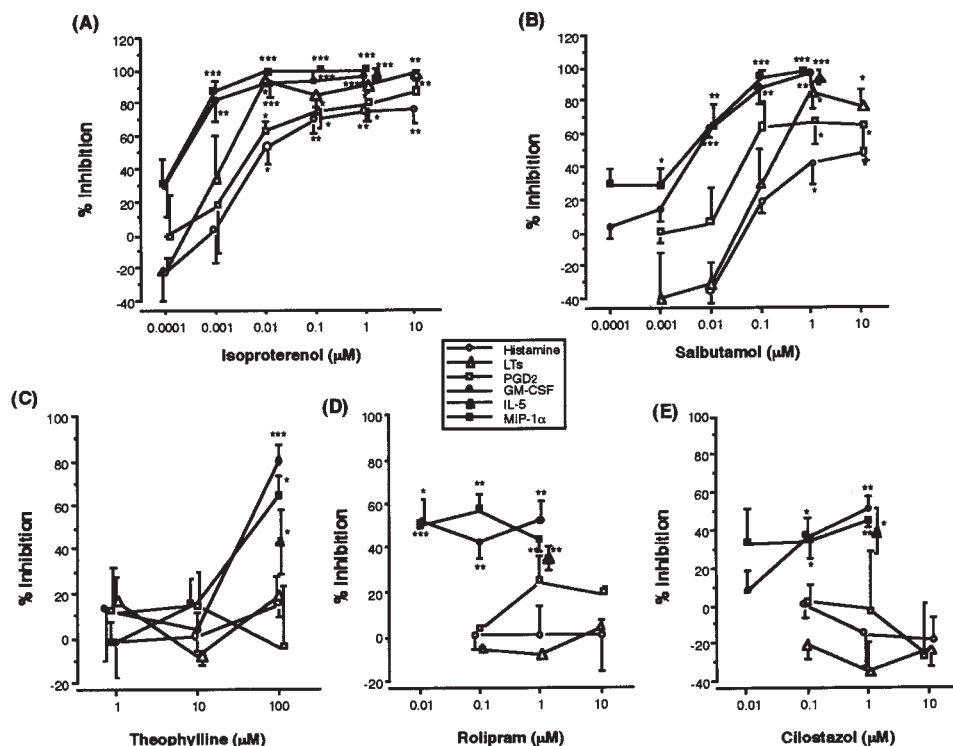


FIG 1. Effects of isoproterenol (A), salbutamol (B), theophylline (C), rolipram (D), and cilostazol (E) on chemical mediator release (histamine, LTs and PGD₂) and on cytokine production (GM-CSF, IL-5, and MIP-1 α) by cultured mast cells. Cells were treated with several concentrations of drugs for 10 minutes and then challenged with anti-IgE at 4 $\mu\text{g}/\text{mL}$ for 30 minutes (chemical mediator release assay) or with anti-IgE at 2 $\mu\text{g}/\text{mL}$ for 360 minutes (cytokine production assay). Values are expressed as the percent inhibition of control chemical mediator release and cytokine production. The mean control histamine, LTs, PGD₂, GM-CSF, IL-5, and MIP-1 α release was $14.3\% \pm 2.3\%$, $2.7 \pm 1.4 \text{ ng}/10^6 \text{ cells}$, $4.3 \pm 1.6 \text{ ng}/10^6 \text{ cells}$, $177.2 \pm 51.3 \text{ pg}/10^6 \text{ cells}$, $581.0 \pm 174.7 \text{ pg}/10^6 \text{ cells}$, and $2.6 \pm 0.8 \text{ ng}/10^6 \text{ cells}$, respectively. Results are the mean $\pm$ SEM of 4 to 6 experiments. Significant differences between control mediator release and drug-treated mediator release: * $P < .05$; ** $P < .01$; *** $P < .001$.

EIA kit (Amersham Int plc, Buckinghamshire, UK) was used to measure the cAMP level. Values were expressed as the percent elevation relative to the control cAMP level at each time.

Statistical analysis

Statistical significance was analyzed by comparing control and treated cells using the Student *t* test for paired data. Differences were considered to be significant at the $P < .05$ level. All data are expressed as the mean $\pm$ SEM. Correlations between the inhibition of histamine release or GM-CSF production and the elevation of cAMP levels were determined by Spearman's correlation coefficients.

RESULTS

Effects of β -agonists and phosphodiesterase inhibitors on chemical mediator release and cytokine production

The β -agonists, isoproterenol (Fig 1, A) and salbutamol (Fig 1, B), inhibited the release of histamine, LTs, and PGD₂ and the production of GM-CSF, IL-5, and MIP-1 α by cultured mast cells in a dose-dependent manner. Inhibition of arachidonic acid-metabolite (LTs and

PGD₂) release by the β -agonists appeared to be greater than that of histamine release, as reported previously.¹³ Moreover, significant inhibition of cytokine productions by isoproterenol and salbutamol was observed at 10- to 100-fold lower concentrations than those causing significant inhibition of histamine release.

Although none of the phosphodiesterase inhibitors had a significant effect on the release of chemical mediators, these drugs significantly inhibited the production of cytokines (Fig 1, C, D, and E). The inhibitory effects of rolipram and cilostazol on GM-CSF and MIP-1 α production reached a plateau at 0.01 to 0.1 $\mu\text{mol}/\text{L}$. Lower concentrations of rolipram and cilostazol had a weaker effect on the production of GM-CSF and MIP-1 α (0.001 $\mu\text{mol}/\text{L}$ rolipram inhibited GM-CSF and MIP-1 α by 29.9% and 37.2%, respectively; 0.0001 $\mu\text{mol}/\text{L}$ rolipram inhibited GM-CSF and MIP-1 α by 12.0% and 10.4%, respectively; 0.001 $\mu\text{mol}/\text{L}$ cilostazol inhibited GM-CSF and MIP-1 α by 7.1% and 18.4%, respectively). Although the inhibitory effect on IL-5 production of phosphodiesterase inhibitors, but not β -agonists, was weaker than

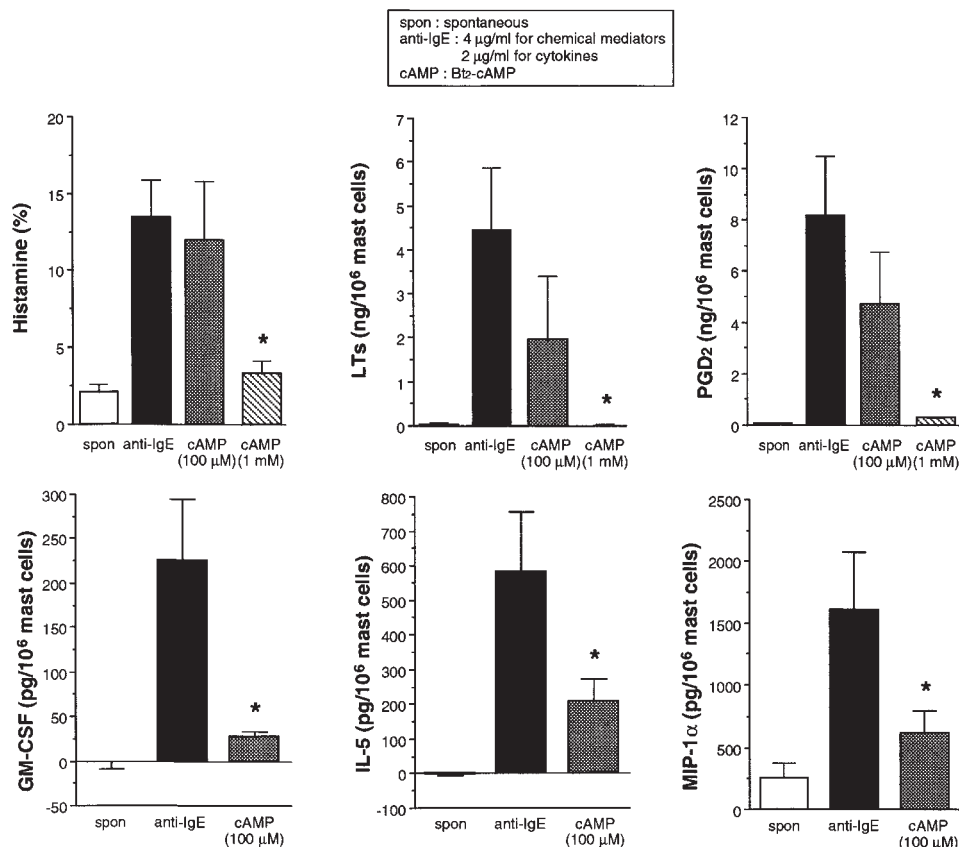


FIG 2. Effect of dibutyl cAMP on anti-IgE-induced release of histamine (A), LTs (B), and PGD₂ (C) and on the production of GM-CSF (D), IL-5 (E), and MIP-1α (F) by cultured mast cells. Cells were treated with dibutyl cAMP at 100 μmol/L or 1 mmol/L for 10 minutes and then challenged with anti-IgE at 4 μg/mL for 30 minutes (chemical mediator release assay) or with anti-IgE at 2 μg/mL for 360 minutes (cytokine production assay). Results are the mean ± SEM of 4 to 5 experiments. Significant differences between control and drug-treated values: **P* < .05.

that on GM-CSF and MIP-1α production, the difference was not significant. These drugs had no effect on the viability of cultured mast cells at 360 minutes after treatment when assessed by the trypan blue exclusion test.

Effect of dibutyl cAMP on chemical mediator release and cytokine production

As shown in Fig 2, 1 mmol/L dibutyl cAMP inhibited the release of histamine, LTs, and PGD₂, although 100 μmol/L dibutyl cAMP inhibited the production of GM-CSF, IL-5, and MIP-1α. These results suggested that intracellular cAMP was involved in regulating the release of chemical mediators and the production of cytokines by mast cells and that cytokine production was more sensitive to cAMP than chemical mediator release.

Kinetics of cAMP elevation by β-agonists and phosphodiesterase inhibitors

To determine whether β-agonists and phosphodiesterase inhibitors affect the cAMP level in cultured mast cells, we treated cells with these drugs in the presence of anti-IgE for 10 to 360 minutes and then measured cAMP. Significant elevation of the cAMP level was caused by

isoproterenol (0.01 and 1 μmol/L; Fig 3, A) and salbutamol (1 μmol/L; Fig 3, B) after 10 minutes of treatment. The cAMP levels declined again at 60 minutes and then rose to reach a plateau at 180 minutes. In contrast, isoproterenol (0.0001 μmol/L; Fig 3, A), and salbutamol (0.0001 and 0.01 μmol/L; Fig 3, B) elevated cAMP levels after 180 minutes of treatment without affecting the level at 10 minutes. In the case of phosphodiesterase inhibitors, a slight elevation of cAMP levels was recognized from 180 minutes after treatment (Fig 3, C). The kinetics of cAMP in spontaneous and anti-IgE treated cells were almost the same; the level increased slightly, but not significantly, after 60 minutes of treatment, and the increase persisted until 360 minutes.

Relationship between inhibition of histamine release and elevation of cAMP by β-agonists

We then examined the relationship between the inhibition of histamine release and the elevation of cAMP levels after 10 minutes of treatment with β-agonists. Isoproterenol (0.01 to 10 μmol/L) and salbutamol (1 to 10 μmol/L) significantly elevated cAMP level (Fig 4, A) in a dose-dependent manner, and the increase corresponded

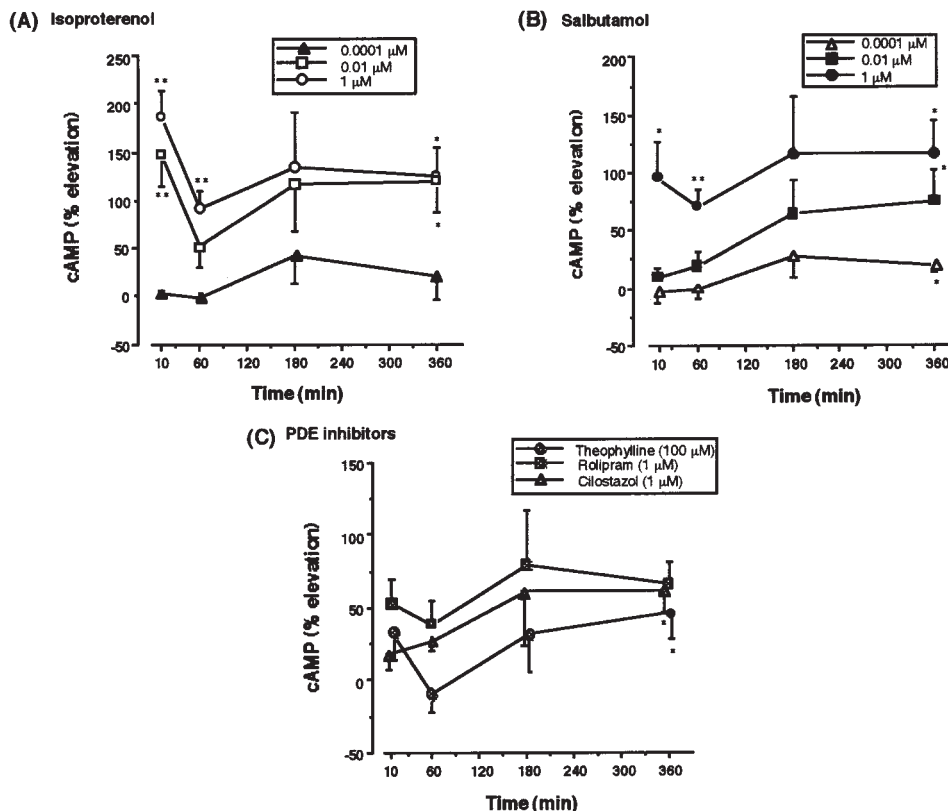


FIG 3. Kinetics of cAMP elevation by β -agonists and phosphodiesterase (PDE) inhibitors. Cells were treated with isoproterenol (A; 0.0001, 0.01, and 1 μ mol/L; $n = 5$), salbutamol (B; 0.0001, 0.01, and 1 μ mol/L; $n = 5$), theophylline (100 μ mol/L), rolipram (1 μ mol/L), and cilostazol (C; 1 μ mol/L; $n = 5$) in the presence of anti-IgE at 2 μ g/mL and then lysed at each specified time. The cAMP level was expressed as the percent elevation of the spontaneous level at each time. The mean spontaneous cAMP level was 40.6 ± 5.2 fmol/ 10^6 cells at 10 minutes, 68.8 ± 4.6 fmol/ 10^6 cells at 60 minutes, 66.6 ± 11.2 fmol/ 10^6 cells at 180 minutes, and 61.4 ± 10.4 fmol/ 10^6 cells at 360 minutes after treatment. Significant differences between spontaneous and drug-treated cAMP levels: * $P < .05$, ** $P < .01$.

with the inhibition of histamine release by these drugs (Fig 1, A and B). There was a strong correlation between the inhibition of histamine release and the elevation of cAMP levels after 10 minutes of drug treatment ($r = 0.73$; $P < .0001$; $n = 20$; Fig 4, B).

Relationship between inhibition of GM-CSF production and elevation of cAMP by β -agonists or by phosphodiesterase inhibitors

We then examined the relationship between the inhibition of GM-CSF production and the elevation of cAMP levels. We measured the amount of GM-CSF at 360 minutes and the amount of intracellular cAMP after 180 minutes of drug treatment in the presence of anti-IgE. The Spearman's correlation coefficient for the relationship between the inhibition of GM-CSF production and the elevation of cAMP by β -agonists, rolipram, cilostazol, and theophylline was 0.73 ($n = 28$), 0.58 ($n = 9$), 0.56 ($n = 9$) and 0.24 ($n = 6$), respectively. As shown in Fig 5, A, there was a significant correlation between the inhibition

of GM-CSF production and the elevation of cAMP by β -agonists, rolipram, and cilostazol ($r = 0.78$; $P < .0001$; $n = 46$). Although comparatively higher concentrations of β -agonists (isoproterenol at 0.01 and 1 μ mol/L and salbutamol at 1 μ mol/L) almost completely inhibited GM-CSF production (94% to 100% inhibition), the cAMP level was elevated by these drugs in a dose-dependent manner (43% to 84% elevation). Theophylline (100 μ mol/L) also inhibited GM-CSF production (mean inhibition, 81.8%; $P < .0001$), whereas cAMP level after 180 minutes of treatment was unchanged (mean elevation, 10.5%; Fig 5, B).

DISCUSSION

In this study, we demonstrated that the inhibition of chemical mediator release and cytokine production by β -agonists and phosphodiesterase inhibitors, except for theophylline, was correlated well with the elevation of intracellular cAMP levels in cultured human mast cells.

It has already been reported that β -agonists inhibit the

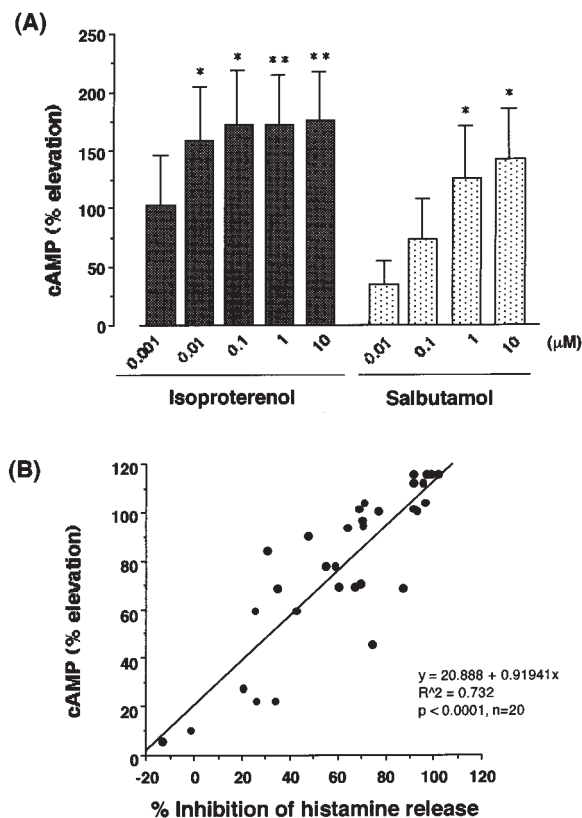


FIG 4. A, Effects of β -agonists, isoproterenol (0.001 to 10 mmol/L) and salbutamol (0.01 to 10 mmol/L), on the cAMP level after 10 minutes of drug-treatment in the presence of anti-IgE (4 μ g/mL). The cAMP level was expressed as the percent elevation of the spontaneous level. The mean spontaneous cAMP level was 51.8 ± 9.6 fmol/ 10^6 cells. Results are the mean $\pm$ SEM of 6 experiments. Significant differences between the spontaneous cAMP and the drug-treated cAMP level: * $P < .05$; ** $P < .01$. B, Relationship between inhibition of histamine release and elevation of cAMP by β -agonists ($n = 20$). Histamine release is expressed as the percent inhibition of control release. The mean control histamine release was $12.3\% \pm 2.3\%$. The relation between the inhibition of histamine release and the elevation of cAMP was determined by Spearman's correlation coefficient.

antigen-induced release of chemical mediators such as histamine, LTs, and PGD_2 from human lung tissue fragments⁸⁻¹² and dispersed human lung mast cells.¹³⁻¹⁵ In the present study, we found that isoproterenol and salbutamol inhibited anti-IgE-induced release of histamine, LTs, and PGD_2 from cultured human mast cells. We also demonstrated that β -agonists suppressed the production of cytokines (GM-CSF, IL-5, and MIP-1 α) induced by anti-IgE stimulation. The mechanism of action of β -agonists appeared to be related to the activation of adenylate cyclase coupled to β -adrenoceptors, which increased intracellular cAMP. Dibutyl cAMP, a lipophilic analog of cAMP, also inhibited chemical mediator release and cytokine production by cultured human mast cells, demonstrating that these processes were downregulated by cAMP. Release of the preformed mediator (histamine)

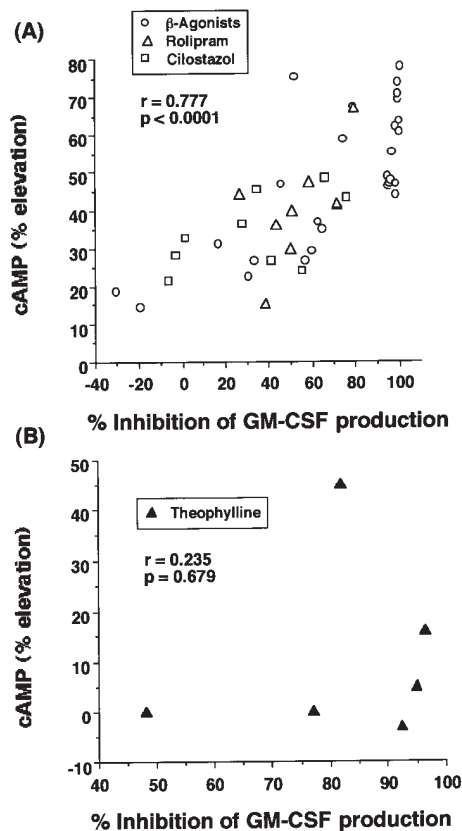


FIG 5. Relationship between inhibition of GM-CSF production and elevation of cAMP by β -agonists or phosphodiesterase inhibitors. Cells were treated for 180 minutes with β -agonists, rolipram, cilostazol, and theophylline in the presence of anti-IgE (2 μ g/mL) and then lysed. The effects of drugs on GM-CSF production are expressed as the percent inhibition of control production. The mean control GM-CSF production was 567.4 ± 176.7 pg/ 10^6 cells. The levels of cAMP are expressed as the percent elevation of spontaneous level. The mean spontaneous cAMP level was 85.4 ± 3.2 fmol/ 10^6 cells. The relation between the inhibition of GM-CSF production and the elevation of cAMP was determined by Spearman's correlation coefficient.

and the newly generated arachidonic acid metabolites (LTs and PGD_2) reached a plateau within 30 minutes, whereas the production of cytokines and chemokines (GM-CSF, IL-5, and MIP-1 α) was recognized from 120 minutes after anti-IgE challenge and reached a plateau at 360 minutes or more after challenge.⁴ Although the role of cAMP in the regulatory pathway for chemical mediator release and cytokine production is not clear, the inhibition of histamine release was well correlated with the elevation of cAMP after 10 minutes of drug treatment, and the inhibition of GM-CSF production was well correlated with the cAMP level at 180 minutes. Interestingly, it has been reported that cAMP-elevating agents inhibited the production of T_{H1} cytokines such as IL-2, but not T_{H2} cytokines such as IL-4 or IL-5 (some studies have shown that these agents enhanced T_{H2} cytokine pro-

duction)^{21,22}; although in the case of cultured mast cells, the elevation of cAMP nonselectively inhibited chemical mediator release and cytokine production. Several authors²³⁻²⁷ have reported that cAMP blocked the immunologically induced increase of Ca²⁺ in rat or mouse mast cells. A possible mechanism of action for cAMP is therefore assumed to be prevention of Ca²⁺ release from intracellular stores²⁸ and/or prevention of Ca²⁺ influx from extracellular fluid.²⁹

It has been reported that nonselective phosphodiesterase inhibitors, such as theophylline and IBMX, effectively block antigen-induced histamine release from rat mast cells^{30,31} and human lung tissue fragments.³² However, the cAMP-selective phosphodiesterase inhibitors (rolipram³³ and RO20-1724³⁰) do not inhibit IgE-mediated histamine release from rat mast cells. There have been few reports about the effects of selective phosphodiesterase inhibitors on human mast-cell-mediator release, and whether these inhibitors suppress mediator release by increasing cAMP levels is unresolved. In the present study, a nonselective phosphodiesterase inhibitor (theophylline), a type IV-selective inhibitor (rolipram), and a type III-selective inhibitor (cilostazol) all failed to affect chemical mediator release or elevate the cAMP level after 10 minutes of treatment. The reason for this discrepancy between the effects of β -agonists and phosphodiesterase inhibitors is not clear. The concentrations of the inhibitors used in the present study were high enough to inhibit phosphodiesterase activity and to induce a significant increase of cAMP levels in human basophils.³⁴ In contrast to the short duration of drug treatment, phosphodiesterase inhibitors (except for theophylline) increased cAMP levels after 180 minutes of treatment and significantly inhibited cytokine production at 360 minutes after challenge. Phosphodiesterase activity in human mast cells may be weak and may be insufficient to rapidly elevate cAMP, but its activity may gradually increase and cause intracellular cAMP levels to rise over a longer period. There have been no reports on the long-term kinetics of cAMP after treatment of mast cells with any agent. In the case of monocytes, the cAMP level peaked within 60 minutes after the addition of phosphodiesterase inhibitors,³⁵ but the reason cAMP increased after a time lag in the present study is not clear. We pretreated mast cells with a protein synthesis inhibitor, cycloheximide, 5 μ g/mL or an RNA synthesis inhibitor (actinomycin D, 5 μ g/mL) before the addition of drugs to determine whether the late increase of cAMP was dependent on new protein synthesis. However, both inhibitors failed to suppress the late elevation of cAMP levels produced by β -agonists and phosphodiesterase inhibitors, suggesting that it was independent of de novo protein synthesis. Interestingly, although the inhibition of GM-CSF production by β -agonists, rolipram, and cilostazol was related to the elevation of intracellular cAMP, the inhibition of GM-CSF production by theophylline was not related. Some other mechanism may be mediated in the effect of theophylline. Further studies concerning a mechanism of theophylline are necessary.

β -Agonists, theophylline, and selective-phosphodi-

esterase inhibitors have been reported to inhibit not only the early but also the late asthmatic response in vivo.³⁶⁻³⁹ The inhibition of mast-cell chemical-mediator release and cytokine production by β -agonists and phosphodiesterase inhibitors may contribute to their inhibition of the asthmatic response. Agents that can cause long-term inhibition of mast-cell chemical-mediator release and cytokine production may be of therapeutic benefit, and cultured human mast cells may be useful for studies on the development of antiasthma agents that inhibit mast-cell function.

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