

The effect of a selective α_2 -adrenergic receptor antagonist (midaglizole) on the cyclic 3',5'-adenosine monophosphate production in human mononuclear cells

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A selective α_2 -adrenergic antagonist, midaglizole, has been recently reported to have efficacy in patients with asthma. To understand the mechanisms, we investigated the effect of midaglizole on the cyclic 3',5'-adenosine monophosphate (cAMP) production in human mononuclear cells (MNCs). MNCs were separated by a histopaque gradient from 10 normal subjects and 10 subjects with asthma. cAMP was measured by RIA kits. Midaglizole (10 $\mu\text{mol/L}$) significantly enhanced isoproterenol-induced cAMP production in both groups, although midaglizole (from 1 to 100 $\mu\text{mol/L}$) did not increase the cAMP production by itself. The percent increase in cAMP was more in subjects with asthma (183.8%) than in normal subjects (140.4%); however, the absolute increase was not different. Platelet-activating factor has been demonstrated to inhibit β -agonist-induced cAMP production in several mammalian tissues, including human MNCs. Midaglizole also prevented platelet-activating factor-induced desensitization of the cAMP response to isoproterenol in MNCs. These findings suggest that midaglizole may be a useful additional agent for the therapy of bronchial asthma through an enhancement of the cAMP production. (J ALLERGY CLIN IMMUNOL 1992;89:722-9.)

Key words: Bronchial asthma, midaglizole, β_2 -adrenergic antagonist, cyclic 3',5'-adenosine monophosphate, mononuclear cells, platelet-activating factor

Although the precise pathophysiologic mechanisms of BA remain unknown, a functional imbalance of α - and β -adrenergic-receptor responses has been considered as one of the fundamental abnormalities in asthma.^{1, 2} The β -adrenergic responsiveness of leukocytes from subjects with asthma is significantly depressed compared with that of normal subjects.^{3, 4} The function of β -adrenergic receptors on the cell surface is likely to be modulated by a number of factors.⁵ Recently, we demonstrated that PAF decreased the density of β -adrenoceptors in the human lung and reduced the responsiveness of β -adrenoceptors to β -agonists in human trachea and MNCs.^{6, 7}

Abbreviations used

cAMP:	Cyclic 3',5'-adenosine monophosphate
MNC:	Mononuclear cell
PAF:	Platelet-activating factor
BA:	Bronchial asthma
Gs protein:	Stimulatory subunit of the guanine nucleotide regulatory protein
Gi protein:	Inhibitory subunit of the guanine nucleotide regulatory protein
HBSS:	Hanks' balanced salt solution

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Szentivanyi et al.⁸ also considered the possibility of hypersensitive α -adrenergic receptors in asthma. There is evidence in some subjects with asthma that the administration of an α -agonist, such as methoxamine, provokes airway narrowing^{9, 10} and that subjects with asthma may respond to treatment with α -adrenergic antagonists, such as phentolamine and thymoxamine.^{11, 12} In contrast, there are some studies that reveal α -antagonists have no effect on pulmonary

function in subjects with asthma.¹³⁻¹⁵ It still remains controversial whether α -adrenergic (especially α_2 -adrenergic) hypersensitivity may play an important role in the pathogenesis of BA. Studies with more selective α_2 -adrenergic-receptor antagonists are necessary to elucidate the exact role of α_2 -adrenergic receptors in asthma.

Midaglizole (2-[2-(4,5-dihydro-1H-imidazol-2-yl)-1-phenylethyl] pyridine dihydrochloride sesquihydrate), a new selective α_2 -adrenergic-receptor antagonist, has been recently developed in Japan.¹⁶ Midaglizole has been introduced as a new oral hypoglycemic drug through its antagonism of α_2 -adrenergic receptors on pancreatic beta cells.¹⁶ Midaglizole has been recently demonstrated to have a bronchodilating effect in moderate to severe BA.¹⁷ To understand the mechanism of the effect of midaglizole in asthma, we investigated the effect of midaglizole on the cAMP production in human MNCs. We also investigated whether midaglizole prevents PAF-induced desensitization of the cAMP response to a β -agonist in MNCs.

MATERIAL AND METHODS

Chemicals

Chemicals were obtained from the following sources: ethylenediaminetetraacetic acid, trichloroacetic acid, ethyl ether, and Wright's stain solution from Fisher Scientific Co., Orangeburg, N.Y.; dextran, Histopaque, Giemsa-stain solution, trypan blue solution, human albumin, 1-epinephrine, and 1-isoproterenol from Sigma Chemical Co., St. Louis, Mo.; HBSS from Gibco Laboratories, Grand Island, N.Y.; Turk blood-diluting fluid from Ricca Chemical Co., Arlington, Texas; C16 PAF from Bachem Laboratory, Inc., Torrance, Calif.; midaglizole from Daiichi Pharmaceutical Co. Ltd., Tokyo, Japan; and ¹²⁵I-labeled cAMP RIA kit from New England Nuclear-DuPont, Boston, Mass.

Subjects

Ten normal healthy subjects (all were male) and 20 subjects with mild asthma (all were male) participated in this study. The mean age of the normal subjects was 27.4 ± 1.2 years (mean $\pm$ SEM), and of the patients with asthma, 30.2 ± 1.4 years. Normal subjects had no history of atopy and took no medications for at least 1 month. All subjects with asthma had a history of recurrent episodic dyspnea, wheezing, and chest tightness that improved with a bronchodilator. Subjects with asthma were all asymptomatic and had not required maintenance β -agonists, corticosteroids, or any antiallergic drugs for at least 2 weeks before their participation in this study. All subjects were selected to exclude hypertension, endocrine diseases, smoking, or psychiatric disorders. All participants refrained from coffee, tea, cola beverages, alcohol, and strenuous exercise for 24 hours before the study. Venous blood was drawn in the morning to avoid circadian changes in cAMP. Informed

consent was obtained from all participants, and this study was approved by the Creighton University Institutional Review Board.

Cell preparation

Forty milliliters of venous blood was drawn into tubes with 1.5% ethylenediaminetetraacetic acid. Blood was incubated with 6% dextran in normal saline for 30 to 60 minutes at room temperature. The plasma-leukocyte fraction was decanted and diluted with HBSS without $\text{Ca}^{++}/\text{Mg}^{++}$ and washed twice by low-speed centrifugation (130 g for 10 minutes at 4°C) to remove platelets. MNCs were isolated by density-gradient centrifugation on Histopaque (density, 1.077) (700 g for 35 minutes at room temperature). MNCs were washed twice in cold HBSS with low-speed centrifugation. The cell count was performed with a hemocytometer with Turk stain, and the cell population was evaluated by cytopspin slides stained with Wright's Giemsa stain. The MNC fraction usually contained about 86% lymphocytes and 12% monocytes, and the polymorphonuclear cell contamination was <2%. The ratio of platelets to MNCs was <0.1. There was no difference in the cell differential and platelet contamination between both groups. The viability of MNCs was >97% with trypan blue dye exclusion method. Finally, MNCs (1×10^7 ml) were resuspended in HBSS with $\text{Ca}^{++}/\text{Mg}^{++}$ and 0.03% human serum albumin.

cAMP assay

Incubations (in duplicate) were performed in 0.5 ml of final volumes in glass tubes at 37°C. The cells were preincubated, with or without, midaglizole (1 to 100 $\mu\text{mol/L}$) for 10 minutes, and then appropriate concentrations of isoproterenol, epinephrine, and/or PAF were added. The incubation was allowed to continue for an additional 10 minutes. The reaction was terminated by addition of 0.5 ml of 12% trichloroacetic acid, followed by centrifugation at 1500 g for 10 minutes at 4°C. The supernatants were purified by ether extraction and kept at -70°C until cAMP assay was performed. cAMP was determined with ¹²⁵I-labeled cAMP RIA kits.

Statistical analysis

All the values are represented as mean $\pm$ SEM. Statistical analysis was performed by an analysis of variance test for unpaired observations or Wilcoxon's test for paired observations; *p* values <0.05 were considered statistically significant.

RESULTS

Although midaglizole (from 1 to 100 $\mu\text{mol/L}$) did not increase the cAMP production by itself in both normal subjects and groups with asthma (Fig. 1), midaglizole at 10 $\mu\text{mol/L}$ significantly enhanced isoproterenol-induced cAMP production in both groups (Fig. 2). With 1 $\mu\text{mol/L}$ of isoproterenol, the cAMP content increased from 11.7 ± 1.3 to 15.2 ± 1.9 pM in normal subjects and from 5.9 ± 1.0 to

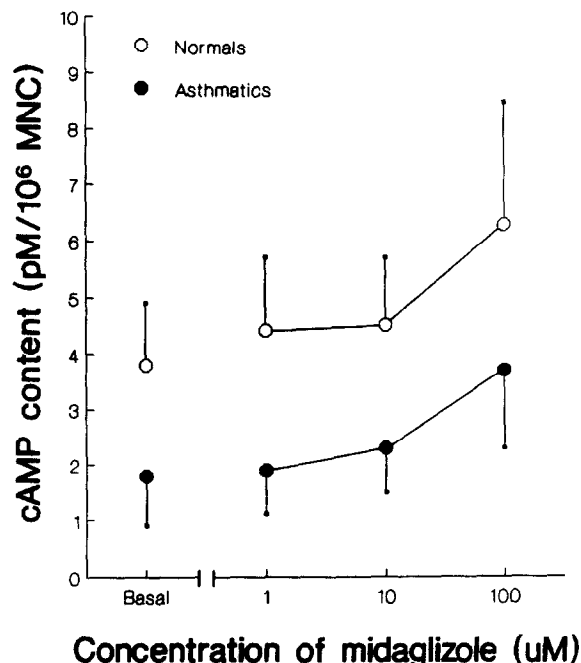


FIG. 1. Effect of midaglizole on cAMP production in human MNCs. No significant increase in cAMP in normal subjects and in subjects with asthma. No statistical difference between the two groups.

9.1 ± 1.7 pM/ 10^6 MNCs in subjects with asthma (Table I). In subjects with asthma the cAMP response to isoproterenol, with or without midaglizole, is lower than in normal subjects, as presented in Table I. This finding is consistent with previous studies.^{3, 4, 7} An enhancement is illustrated in Fig. 3 of the cAMP production by midaglizole in the presence of isoproterenol, which was obtained by subtraction of the cAMP production induced by midaglizole alone. In the presence of 1 μ mol/L of isoproterenol, midaglizole significantly enhanced the cAMP production in a dose-dependent manner in both groups.

The cAMP production induced by midaglizole in the absence or presence of isoproterenol was obtained by subtraction of the basal cAMP level (Fig. 4). Midaglizole alone (10 μ mol/L) increased cAMP of 0.8 ± 0.5 pM in normal subjects, and 0.5 ± 0.3 pM in subjects with asthma. When midaglizole was combined with isoproterenol, however, a synergistic effect on the cAMP production was observed in both groups. In subjects with asthma the cAMP production increased from 4.0 ± 0.6 to 7.2 ± 1.1 pM, and in normal subjects, from 8.1 ± 1.0 to 11.5 ± 1.3 pM. The value of percent increase on the cAMP production in subjects with asthma ($183.8\% \pm 18.9\%$) was significantly higher than in normal subjects ($140.4\% \pm 13.8\%$). Midaglizole also enhanced epi-

nephine-induced cAMP production synergistically (Table II). Although midaglizole had a tendency to enhance cAMP accumulation induced by epinephrine more than by isoproterenol, it did not reach statistical significance.

We have recently reported that PAF prevented the cAMP response to isoproterenol in human MNCs.⁷ As illustrated in Fig. 5, PAF at 0.1 and 1 μ mol/L significantly decreased isoproterenol-induced cAMP production in MNCs from normal subjects and subjects with asthma. There was no significant difference in the response to PAF between normal subjects and subjects with asthma. Midaglizole, at 10 and 100 μ mol/L, prevented PAF (1 μ mol/L)-induced desensitization of the cAMP response to isoproterenol in MNCs from both groups (Table III). In subjects with asthma, midaglizole, at 10 and 100 μ mol/L, significantly ($p < 0.05$) increased isoproterenol-induced cAMP production, even in the presence of PAF. In contrast, normal subjects had a significant increase in cAMP at 100 μ mol/L of midaglizole in the presence of PAF. This finding suggested that subjects with asthma respond to midaglizole at lower concentrations than normal subjects.

DISCUSSION

Several investigators have demonstrated the efficacy of α -adrenergic-receptor antagonists, such as phentolamine and thymoxamine, in patients with exercise-induced or allergen-induced BA.^{11, 12} The combination of isoproterenol and either phentolamine or thymoxamine produced marked improvement in pulmonary function, significantly more improvement than isoproterenol alone.^{18, 19} In support of these clinical findings are the observations that phentolamine or thymoxamine enhanced the cAMP response to isoproterenol in leukocytes from normal subjects and subjects with asthma.^{20, 21} In contrast, there are some studies that reveal α -antagonists were not effective in subjects with asthma.¹³⁻¹⁵ According to the observation by Spector,¹³ when phentolamine was administered by inhalation to 68 subjects with asthma, only 16% had bronchodilatation and 12% had bronchoconstriction. These clinical and experimental findings suggest the existence of α -adrenergic hyperresponsiveness in a subset of the population with asthma and suggest α -receptor blockade may improve the clinical condition of subjects with asthma through an elevation of the cAMP production. When the efficacy of α -adrenergic antagonists are considered, as described above, these α -antagonists may have other properties, such as α_1 -antagonistic and antihistamine activity, in addition to α_2 -antagonistic activity. To clarify the significance of α_2 -adrenoceptor mechanisms in asthma,

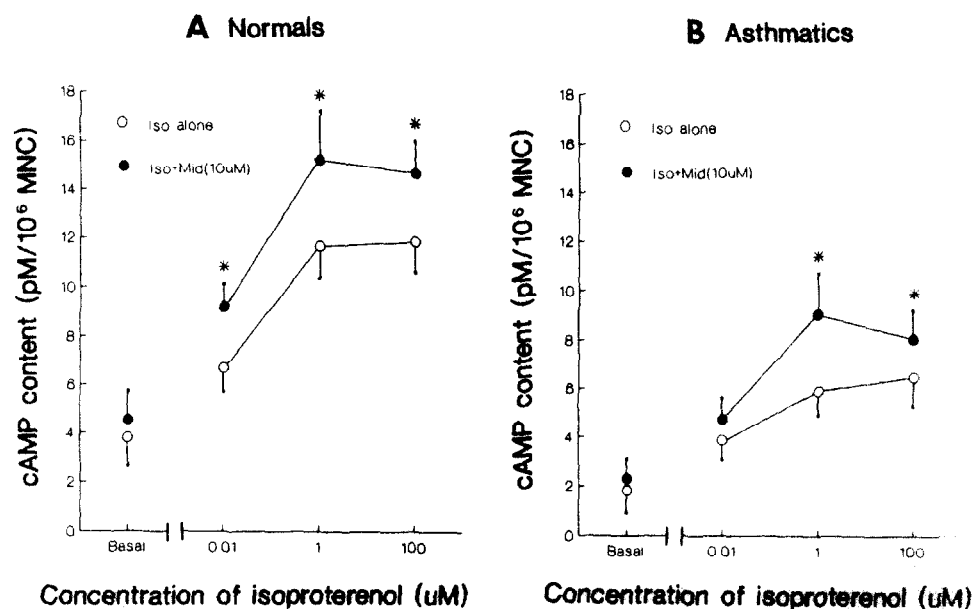


FIG. 2. A, Effect of combination of midaglizole and isoproterenol on cAMP production in MNCs from normal subjects. **B,** Effect on cAMP production in MNCs from subjects with asthma. Concentration of midaglizole used was 10 $\mu\text{mol/L}$; * $p < 0.05$, compared with value in control group (isoproterenol alone) by Wilcoxon's test.

TABLE I. Summary of cAMP production in MNCs induced by isoproterenol and midaglizole

Conditions	cAMP content (pM/10 ⁶ MNCs)		<i>p</i> Value*
	Subjects		
	Normal (N = 10)	With asthma (N = 10)	
Basal value	3.7 ± 1.2	1.8 ± 0.9	NS
Iso			
1 μmol/L	11.7 ± 1.3	5.9 ± 1.0	<0.01
100 μmol/L	11.8 ± 1.3	6.5 ± 1.2	<0.02
Mid 10 μmol/L	4.5 ± 1.2†	2.3 ± 0.8†	NS
Mid plus Iso 1 μmol/L	15.2 ± 1.9‡	9.1 ± 1.7§	<0.05
Mid plus Iso 100 μmol/L	14.6 ± 1.3‡	8.1 ± 1.2§	<0.01

Iso, Isoproterenol; Mid, midaglizole; NS, not significant.

Compared with the value induced by isoproterenol alone in the same group by Wilcoxon's test:

* p values were obtained by analysis of variance test.

[†] p , not significant, compared with a basal value by Wilcoxon's test.

[‡] $p < 0.05$.

[§] $p < 0.02$.

more preferential and specific α_2 -antagonists have been developed.

Midaglizole is demonstrated to be a specific α_2 -adrenergic-receptor antagonist through studies on insulin release from pancreatic islets and radioligand binding assay in rat cerebral cortex membranes.^{16, 22} Midaglizole is a more preferential and specific α_2 -

antagonist than yohimbine and lacks antihistamine activity.^{22, 23} When midaglizole was combined with isoproterenol, midaglizole significantly enhanced the bronchodilating effect of isoproterenol.²³ Midaglizole was also effective in patients with moderate to severe BA when it was added to conventional treatment.¹⁷

In this study, we demonstrated that midaglizole en-

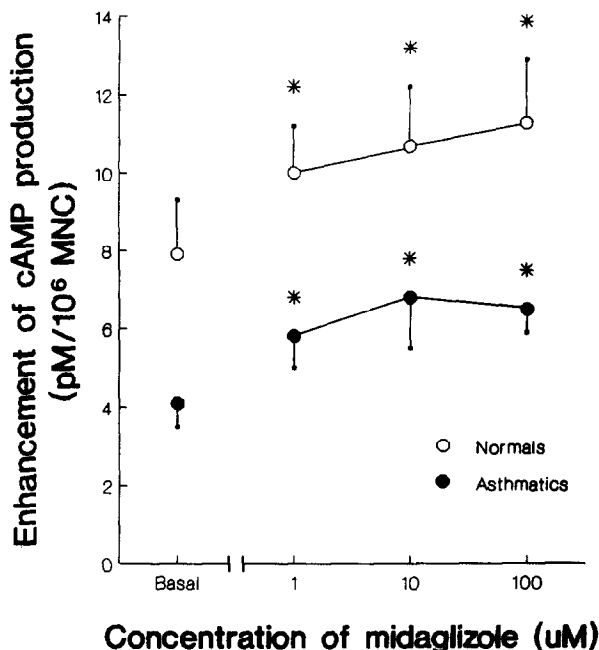


FIG. 3. Enhancement of cAMP production by midaglizole in the presence of isoproterenol in normal subjects and subjects with asthma. Concentration of isoproterenol used was 1 $\mu\text{mol/L}$. cAMP increase by midaglizole was obtained by subtraction of cAMP production induced by midaglizole alone; * $p < 0.05$, compared with basal value without midaglizole by Wilcoxon's test. Significant differences ($p < 0.05$) between two groups at all concentrations of midaglizole by analysis of variance test.

hanced isoproterenol-induced cAMP production in human MNCs from normal subjects and subjects with asthma, although it did not increase the cAMP production by itself. Moreover, this synergistic effect of midaglizole on the cAMP production resulted in a higher percent increase in subjects with asthma than in normal subjects. This synergistic effect of midaglizole was also found on epinephrine-induced cAMP production. We expected that midaglizole would be more potent on epinephrine-induced cAMP production than on isoproterenol-induced cAMP production because epinephrine is known to have more α_2 -adrenergic activity. Although midaglizole had a tendency to enhance cAMP accumulation induced by epinephrine more than that induced by isoproterenol, we could not find any significant differences between these two adrenergic agents. However, Sakai et al.²⁴ recently reported that BHT-933 (specific α_2 -adrenergic agonist) suppressed isoproterenol-induced cAMP production from human leukocytes and that midaglizole prevented the effect of BHT-933 on isoproterenol-induced cAMP production. This study suggests that the synergistic effect of midaglizole on cAMP

production is mediated via α_2 -adrenergic receptors. The synergistic effect on cAMP production is also demonstrated with another α -antagonists, such as phentolamine and thymoxamine.^{20, 21} The effect of phentolamine might be due to β -agonistic activity as well as an α -blockade.²⁰ However, we considered α -blockade as the main reason for the synergistic effect of midaglizole because midaglizole has been demonstrated not to have β -agonistic activity.²⁵

In considering the effect of α -antagonists on the cAMP production in MNCs, the problem of platelet contamination in the MNC fraction is significant. Lad et al.²⁶ reported that there was no α_2 -receptor binding in MNCs from normal subjects, although low levels of α_2 -adrenoceptor binding were found in MNCs from subjects with mild asthma with highly purified MNCs (platelet contamination, <1%). It has been reported that there was no difference in density and affinity of platelet α_2 -receptors or in platelet cAMP responses to stimulation by α -agonists between normal subjects and subjects with asthma.^{7, 27} Although the platelet contamination in the MNC fraction was <9% in this study, platelets may play a role in the effect of midaglizole on the cAMP production in MNCs from normal subjects. The finding that a synergistic effect of midaglizole on isoproterenol-induced cAMP production resulted in a higher percent increase in subjects with asthma than in normal subjects may, however, be accounted by the presence of increased or hypersensitive α_2 -adrenoceptors in MNCs from subjects with asthma, compared with that of normal subjects.

The adenylate cyclase system that produces cAMP in cell surface is regulated by both a stimulating factor (Gs protein) and an inhibitory factor (Gi protein).²⁸ Activation of α_2 -adrenergic receptors inhibits the catalytic unit of adenylate cyclase via activation of Gi protein (release of Gi α -subunits). Therefore, α_2 -adrenergic-receptor antagonists are suggested to act on adenylate cyclase through α_2 -adrenergic receptors and Gi protein. The exact mechanism by which an α -blockade results in the enhancement of isoproterenol-induced cAMP production in MNCs remains unknown. It has been postulated, however, that there may be a direct competition between Gs and Gi α -subunits on the adenylate cyclase and that α_2 -antagonists might result in the enhancement of the cAMP synthesis via inhibition of α_2 -receptors and Gi α -subunits release.⁵ Additional studies are necessary to investigate the exact mechanism of the synergistic effect of α_2 -antagonists.

We also demonstrated in this study that midaglizole prevented PAF-induced desensitization of the cAMP

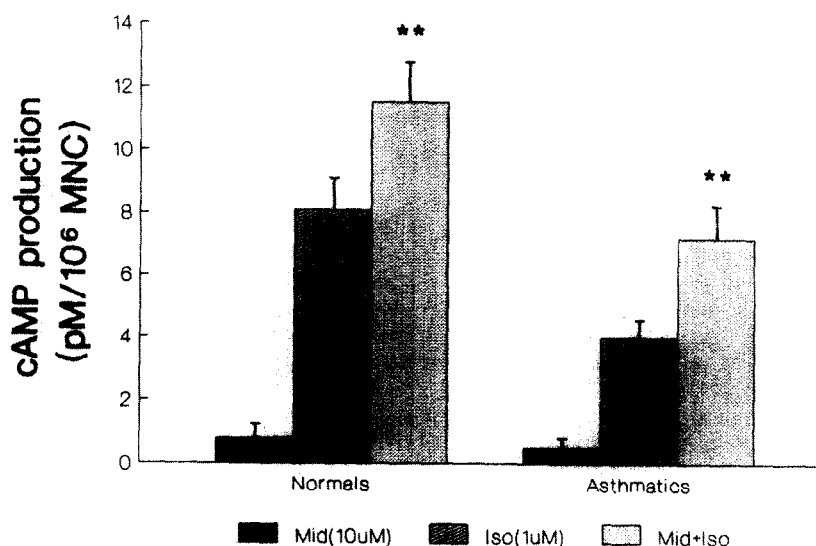


FIG. 4. Effect of midaglizole on isoproterenol-induced cAMP production in MNCs. Concentrations of midaglizole and isoproterenol used were 10 and 1 $\mu\text{mol/L}$, respectively. Each value was subtracted by a basal cAMP level; ** $p < 0.01$, compared with value induced by isoproterenol alone in same group by Wilcoxon's test. Values of percent increase on cAMP production in subjects with asthma and in normal subjects were $183.8\% \pm 18.9\%$ and $140.4\% \pm 13.8\%$, respectively ($p < 0.02$, by analysis of variance test).

response to isoproterenol in MNCs from normal subjects and subjects with asthma. PAF has been demonstrated to inhibit the prostaglandin E_1 -induced rise in cAMP in rabbit platelets,²⁹ prostaglandin E_2 - and salbutamol-induced cAMP production in guinea pigs alveolar macrophages,³⁰ and isoproterenol-induced cAMP production in human MNCs.⁷ α -Adrenergic antagonists, phentolamine and phenoxybenzamine, are reported to specifically inhibit PAF-induced platelet and neutrophil aggregation and degranulation.³¹ Recently, Agrawal et al.³² demonstrated the decrease of the PAF binding to human blood eosinophils by pretreatment with pertussis toxin. These findings suggest that there may be a close relationship between PAF receptors and α_2 -adrenergic receptors (and Gi protein), although the role of guanine nucleotides in PAF-receptor regulation remains unclear. Additional studies will be needed to clarify the mechanisms of interaction between α_2 -adrenoceptors and PAF receptors.

In summary, we demonstrated that midaglizole, a specific α_2 -antagonist, enhanced isoproterenol-induced cAMP production in human MNCs from normal subjects and subjects with asthma, although it did not increase the cAMP production by itself. This synergistic effect of midaglizole resulted in a higher percent increase in subjects with asthma than in normal subjects. Midaglizole also prevented PAF-induced desen-

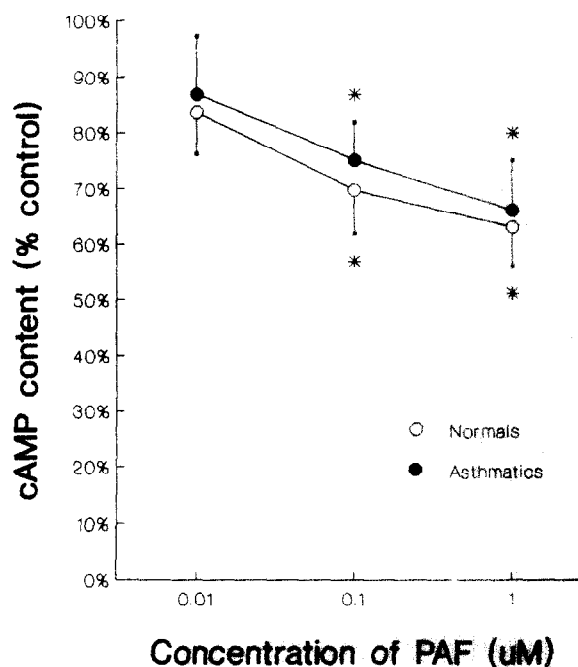


FIG. 5. Effect of PAF on isoproterenol-induced cAMP response in MNCs from normal subjects ($N = 7$) and subjects with asthma ($N = 6$). cAMP content induced by 1 $\mu\text{mol/L}$ of isoproterenol was taken as a 100%, and all other values were calculated as a percent of this cAMP content; * $p < 0.05$, compared with control (isoproterenol alone) by Wilcoxon's test. No significant differences on response to PAF between two groups.

TABLE II. Synergistic effect of midaglizole on epinephrine- and isoproterenol-induced cAMP production in MNCs from subjects with asthma

Conditions	cAMP production (pM/10 ⁶ MNCs)	
	Isoproterenol (1 μ mol/L, N = 10)	Epinephrine (1 μ mol/L, N = 8)
Stimulant alone	4.0 $\pm$ 0.6	5.0 $\pm$ 1.4
Mid (10 μ mol/L) plus stimulant	7.2 $\pm$ 1.1*	8.9 $\pm$ 2.2†
% Increase	183.8% $\pm$ 18.9%	189.0% $\pm$ 15.9%

Mid, Midaglizole.

Value of stimulant-induced cAMP production was obtained by subtraction of a basal cAMP level.

No significant differences between the two stimulants by analysis of variance test.

Compared with the value induced by stimulant alone in the same group by Wilcoxon's test:

* $p < 0.01$.

† $p < 0.02$.

TABLE III. Effect of midaglizole on PAF-induced desensitization of cAMP response to isoproterenol in MNCs from normal subjects and subjects with asthma

Conditions	cAMP content (% control)	
	Subjects	
	Normal (N = 7)	With asthma (N = 6)
PAF (1 μ mol/L)	63.3 $\pm$ 7.0	66.4 $\pm$ 8.9
PAF plus Mid 10 μ mol/L*	96.0 $\pm$ 11.8	†118.1 $\pm$ 5.2†
PAF plus Mid 100 μ mol/L*	133.0 $\pm$ 12.9‡	†145.0 $\pm$ 9.2‡

cAMP content induced by 1 μ mol/L of isoproterenol was taken as a 100%, and all other values were calculated as a percent of it.

* $p < 0.02$.

† $p < 0.05$.

‡ $p < 0.05$, compared with the control (isoproterenol alone) by Wilcoxon's test. There were no significant differences between the two groups by analysis of variance test.

sitization of the cAMP response to isoproterenol in MNCs. These findings suggest that midaglizole may be a useful additional agent in BA through an enhancement of the cAMP production.

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