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Relaxant effect of YM976, a novel phosphodiesterase 4 inhibitor, on bovine tracheal smooth muscle

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Abstract

Effects of 4-(3-chlorophenyl)-1,7-diethylpyrido[2,3-*d*]pyrimidin-2(1*H*)-one (YM976), a novel and selective phosphodiesterase type 4 inhibitor, on tension and adenosine 3′,5′-cyclic monophosphate (cAMP) content of bovine tracheal smooth muscle were compared with those of rolipram and theophylline. YM976, rolipram and theophylline relaxed the tracheal preparations contracted with histamine in a concentration-dependent manner. The relaxant effects of YM976 and rolipram were more potent than those of theophylline. These phosphodiesterase inhibitors-induced relaxations were dramatically diminished when tracheal smooth muscle was contracted with methacholine instead of histamine. Pretreatment of the tracheal preparations with YM976 (10 μM) or rolipram (10 μM), but not with theophylline (1 mM), shifted the concentration–response curves for contractile responses to histamine; however, the same procedure failed to affect concentration–response relationships for methacholine-induced contractions. At 1 and 10 μM, both YM976 and rolipram increased the tissues cAMP content. These results suggest that YM976 relaxes tracheal smooth muscle, probably through the cAMP-dependent mechanism.

Keywords: cAMP; Phosphodiesterase type 4; Relaxation; Smooth muscle, Tracheal

1. Introduction

The cyclic nucleotide phosphodiesterases configure a family of enzymes that hydrolyze adenosine 3′,5′-cyclic monophosphate (cAMP) and guanosine 3′,5′-cyclic monophosphate (cGMP). These enzymes, therefore, regulate multiple signaling pathways through changing intracellular cyclic nucleotide levels. Eleven family members of phosphodiesterase are distributed heterogeneously in different tissues and they differ in substrate specificity, affinity for cyclic nucleotides and regulatory properties (Fawcett et al., 2000; Fisher et al., 1998a,b; Fujishige et al., 1999; Hayashi et al., 1998; Hetman et al., 2000; Loughney et al., 1999; Soderling et al., 1999). Among these enzymes, type 4 phosphodiesterase is a high-affinity cAMP-selective isozyme (Beavo, 1995). The inhibition of phosphodiesterase 4 relaxes airway smooth muscle (Torphy et al., 1988; Harris et al., 1989; Shahid et al., 1991; Heaslip et al., 1994) and induces anti-inflammatory effects, such as the reduction of chemical mediator release from mast cells (Weston et al., 1997), basophils (Peachell et al., 1992; Weston et al., 1997), eosinophils (Dent et al., 1991; Underwood et al., 1993, 1994; Raeburn et al., 1994) and macrophages (Germain et al., 1998; Goncalves de Moraes et al., 1998). As a result, phosphodiesterase 4 inhibitors have been focused as drugs for treatment of asthma and a wide range of inflammatory diseases.

Several selective phosphodiesterase 4 inhibitors, including rolipram, *R*-(+)-4-[2-(3-cyclopentoxy-4-methoxyphenyl)-2-phenylethyl] pyridine (CDP840) (Holbrook et al., 1996) and 3-cyclopentyloxy-*N*-(3,5-dichloro-4-pyridyl)-4-methoxybenzamide (RP73401) (Raeburn et al., 1994) have been developed, and clinical trials showed the potential use of phosphodiesterase 4 inhibitors in asthma and chronic obstructive pulmonary disease (Torphy et al., 1999); however, these phosphodiesterase 4 inhibitors have adverse actions such as nausea and vomiting. In many cases, these undesirable effects limit clinical usefulness of phosphodiesterase 4 inhibitors. Therefore, phosphodiesterase 4 inhibitors with little or no emetic effect have been desired for a novel anti-asthmatic or anti-inflammatory agent. 4-(3-Chlorophenyl)-1,7-diethylpyrido[2,3-*d*]pyrimidin-2(1*H*)-one (YM976) is a novel phosphodiesterase 4

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inhibitor, which is a pyrimidine derivative and totally different from rolipram in structure. This compound showed the dissociation of anti-inflammatory activities from emetic effects (Aoki et al., 2000) and inhibited the antigen-induced airway responses (Aoki et al., 2001b). However, YM976, unlike rolipram and RP73401, exerted only a small relaxant effect on guinea pig isolated tracheal preparations and showed the slight inhibition for bronchospasm induced by leukotriene D_4 and acetylcholine in vivo (Aoki et al., 2001b). The cellular heterogeneity of guinea pig tracheal preparations and in vivo conditions preclude an evaluation of the effects of compounds on tracheal smooth muscle. Thus, we chose to examine the effects of YM976 on relaxation and cAMP accumulation of bovine tracheal smooth muscle. These preparations have been demonstrated to consist ~95% of smooth muscle (Katsuki and Murad, 1977) and are more amenable to the evaluation of second messengers involved in drug-induced relaxation.

2. Materials and methods

2.1. Preparation of bovine tracheal smooth muscle segments

Fresh bovine tracheas were obtained from a local abattoir and transported to the laboratory in ice-cold Krebs–Ringer bicarbonate buffer (composition in mM: NaCl, 118.5; KCl, 4.47; $MgSO_4$, 1.18; KH_2PO_4, 1.18; $CaCl_2$, 2.54; $NaHCO_3$, 24.9; glucose, 10.0; and pyruvic acid, 1.0) (pH = 7.4). The smooth muscle layers were dissected from the cartilage, mucosa and connective tissues while immersed in ice-cold Krebs–Ringer bicarbonate buffer gassed with 95% O_2–5% CO_2. Segments ($1 \times 2 \times 10$ mm) of smooth muscle were used for experiments.

2.2. Measurement of mechanical activity

One end of each muscle was attached by a cotton thread to a force displacement transducer (model TB-611T, Nihon Kohden, Tokyo, Japan) and the other end was tied to a stainless-steel holder. Muscle tension was recorded isometrically. The muscle segments were mounted in 20-ml jacketed organ baths containing Krebs–Ringer bicarbonate buffer gassed with 95% O_2–5% CO_2 at 37 °C, and subsequently allowed to equilibrate for 2 h under an initial tension of 0.75 g. The bath solution was changed every 15 min during the incubation period. The resting tension was adjusted to 0.5 g 10 min before starting each experiment.

2.3. Reversal effects of YM976, rolipram and theophylline on contractile responses to histamine and methacholine

Contractile responses to histamine and methacholine in bovine tracheal smooth muscle preparations were determined in preliminary studies by cumulative addition of agonists (Fig. 1). We examined the reversal effects of YM976, rolipram and theophylline on preparations contracted with two different concentrations of histamine (3 or 100 μM) or methacholine (0.1 μM). After confirming the plateau of the effects of histamine and methacholine, we added cumulatively YM976 (0.1 nM to 10 μM), rolipram (0.1 nM to 10 μM) or theophylline (0.1 nM to 10 μM) to the tissue baths. In separate experiments, we examined the effect of cimetidine, a blocker of histamine H_2 receptors, on relaxant responses to YM976 and rolipram in histamine (100 μM)-contracted bovine tracheal smooth muscle.

2.4. Preventive effects of YM976, rolipram and theophylline on contractile responses to histamine and methacholine

To examine the preventive effects of YM976, rolipram and theophylline on contractile responses to histamine and methacholine, we incubated the tissues with YM976 (0.1 or 10 μM), rolipram (0.1 or 10 μM), theophylline (10 or 1000 μM) or their solvent for 10–15 min and then added histamine (0.1 μM to 10 mM) or methacholine (0.3 nM to 300 μM) to the tissue baths.

2.5. Time-courses of YM976-, rolipram- and theophylline-induced cAMP accumulation

Each smooth muscle segment was equilibrated for 2 h in an organ chamber that warmed to 37 °C and filled with 5 ml of Krebs–Ringer bicarbonate buffer gassed with 95% O_2–5% CO_2. After the equilibration period, tissues were exposed to maximally effective concentration of either YM976, rolipram or theophylline for 3, 5 or 15 min. The concentrations of YM976, rolipram and theophylline were 10 μM, 10 μM and 1 mM, respectively. At the end of the incubation, the tissues were rapidly frozen in liquid nitrogen and stored at −80 °C.

2.6. Concentration-dependencies of YM976-, rolipram- and theophylline-induced cAMP accumulation

To examine the concentration–response relationships for YM976-, rolipram- and theophylline-induced cAMP accu-

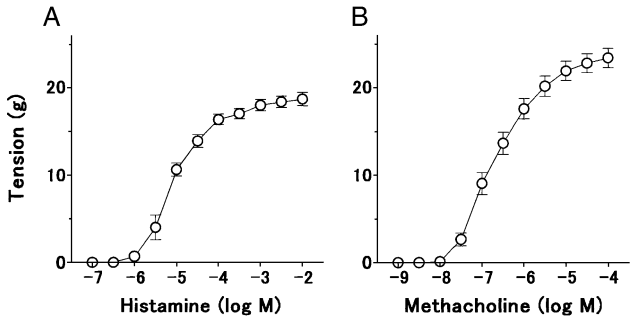

Fig. 1. Effects of histamine and methacholine on the tension of bovine tracheal smooth muscle preparations. Each point with a vertical bar represents the mean ± S.E.M. from five separate preparations.

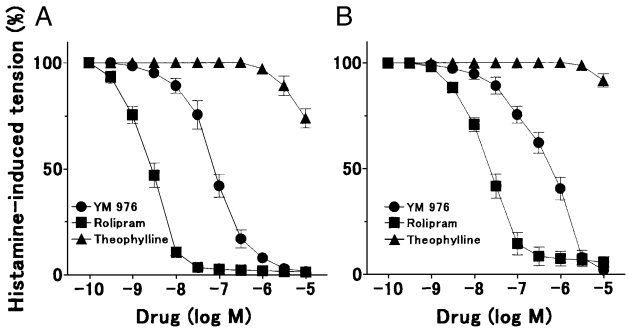

Fig. 2. Reversal effects of YM976, rolipram and theophylline on bovine tracheal smooth muscle preparations precontracted with histamine (A: 3 μM, B: 100 μM). Each point with a vertical bar represents the mean ± S.E.M. from five to six separate preparations.

mulation, we incubated the tissues with YM976 (0.1, 1 or 10 μM), rolipram (0.1, 1 or 10 μM) or theophylline (10, 100 or 1000 μM) for 15 min before freezing for measurement of cAMP content.

2.7. Measurement of tissue cAMP content

Frozen tissue samples were homogenized in 2 ml of ice-cold 6% trichloroacetic acid, using a glass homogenizer. The homogenate was centrifuged at 1500 × *g* for 10 min at 4 °C. The supernatant was extracted three times with three volumes of diethyl ether. We determined the cAMP content using a method of radioimmunoassay (cAMP assay kit, Yamasa Shoyu, Choshi, Japan). The tissues residue was dissolved in 2N NaOH and the protein content was determined using a protein assay kit (Bio-Rad protein Assay, Bio-Rad Laboratories, Hercules, CA, USA) with bovine serum albumin as the standard. Tissue contents of cAMP were presented as pmol/mg protein.

2.8. Data analysis and statistics

Data were expressed as the mean ± S.E.M. pEC_{50} (negative logarithm of the molar concentration of the agonist producing 50% of the corresponding agonist-induced maximum response) and pIC_{50} values (negative logarithm of the molar concentration required to decrease methacholine- or histamine-induced tension by 50%) were determined by nonlinear curve fitting using the GraphPad Prism program (GraphPad Software, San Diego, CA, USA). The data were analyzed using either Student's *t*-test or Dunnet's multiple comparison test after one-way analysis of variance (one-way ANOVA). A *P*-value smaller than 0.05 was considered significant.

2.9. Chemicals

YM976 and rolipram were synthesized by Yamanouchi Pharmaceutical (Tsukuba, Ibaraki, Japan). Acetyl-β-methylcholine chloride (methacholine), calbamylcholine chloride (carbachol), histamine dihydrochloride and theophylline were obtained from Sigma (St. Louis, MO, USA). YM976 was dissolved in ethanol and diluted by Krebs–Ringer bicarbonate buffer. Rolipram was dissolved in dimethyl sulfoxide (DMSO).

3. Results

3.1. Effects of histamine and methacholine

Histamine (0.1 μM–10 mM) and methacholine (0.001–300 μM) produced a concentration-dependent contraction of bovine tracheal smooth muscle preparations with pEC_{50} values of 5.00 ± 0.11 and 6.63 ± 0.08, respectively ($n = 5$) (Fig. 1). The maximum developed tensions of histamine and methacholine were 18.7 ± 0.8 and 23.5 ± 1.1 g, respectively ($n = 5$). Thus, methacholine was both more potent and more effective than histamine.

3.2. Reversal effects of YM976, rolipram and theophylline on histamine- and methacholine-induced contractions

Fig. 2 and Table 1 show the effects of YM976, rolipram and theophylline on histamine-contracted preparations. YM976, rolipram and theophylline concentration-dependently reversed contraction with either 3 or 100 μM histamine (Fig. 2). The relaxant responses to YM976, rolipram and theophylline were reduced substantially as the concentration of histamine was increased. YM976 was less potent than rolipram in attenuating histamine-induced contraction at the concentration range below 1 μM. On the other hand, at 3 and 10 μM, both YM976 and rolipram exhibited almost equal inhibitions against histamine-induced contraction.

Cimetidine did not affect relaxant responses to YM976 and rolipram in the histamine (100 μM)-contracted preparations (pIC_{50} values for YM976, 6.48 ± 0.12 vs. 6.53 ± 0.14; pIC_{50} values for rolipram, 7.80 ± 0.04 vs. 7.85 ± 0.15) ($n = 4$ in each case).

Table 1
Reversal effects of YM976, rolipram and theophylline on bovine tracheal smooth muscle contracted with histamine (3 and 100 μM)

	Histamine (3 μM)		Histamine (100 μM)	
	pIC_{50}	Residual tension (%)	pIC_{50}	Residual tension (%)
YM976	7.14 ± 0.09	1.4 ± 0.4	6.23 ± 0.11[a]	1.8 ± 0.8
Rolipram	8.57 ± 0.08[b]	1.2 ± 0.3	7.62 ± 0.08[a,b]	5.4 ± 2.6
Theophylline	–	73.6 ± 4.4	–	91.5 ± 3.1

The values represent the mean ± S.E.M. from five separate preparations. pIC_{50} indicates negative logarithm of the molar concentration required to decrease methacholine- or histamine-induced tension by 50%. Residual tension is response remaining after administration of maximal concentration (10 μM) of each drug.

[a] $P < 0.01$ vs. corresponding histamine (3 μM) values (unpaired *t*-test).

[b] $P < 0.01$ vs. corresponding YM976 values (unpaired *t*-test).

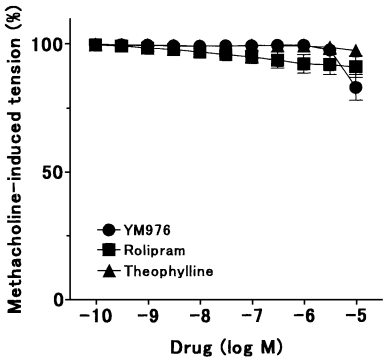

Fig. 3. Reversal effects of YM976, rolipram and theophylline on bovine tracheal smooth muscle preparations precontracted with methacholine (0.1 μM). Each point with a vertical bar represents the mean ± S.E.M. from five to six separate preparations.

Fig. 3 shows the effects of YM976, rolipram and theophylline on contraction with methacholine (0.1 μM). Thus, the relaxant effects of phosphodiesterase inhibitors on methacholine-induced contractions were much smaller than those on histamine (3 or 100 μM)-induced ones.

3.3. Preventive effects of YM976, rolipram and theophylline on histamine- and methacholine-induced contractions

Fig. 4 and Table 2 show the preventive effects of YM976, rolipram and theophylline on histamine-induced contractions. Both YM976 (10 μM) and rolipram (10 μM) significantly shifted the concentration–response curves for histamine-induced contractions to the right by about two-fold without reducing the maximal response. At 0.1 μM, they failed to decrease the pEC_{50} values for histamine-induced contractions (Table 2). In contrast to YM976 and rolipram, theophylline (10 μM and 1 mM) did not significantly affect contractile responses to histamine.

On the other hand, YM976 (10 μM) and rolipram (10 μM) did not shift the concentration–response curves for methacholine-induced contractions (pEC_{50}s; control, 6.72 ± 0.08 vs. YM976, 6.58 ± 0.19; control, 6.55 ± 0.03 vs. rolipram, 6.50 ± 0.09) ($n = 6$ in each case).

Table 2
Preventive effects of YM976, rolipram and theophylline on the contractile response to histamine in bovine tracheal smooth muscle

PDE inhibitor (concentration)	pEC_{50}		E_{max} (%)	
	Control	Treated	Control	Treated
YM976 (0.1 μM)	4.96 ± 0.05	4.84 ± 0.08	76.8 ± 2.3	75.6 ± 3.6
YM976 (10 μM)	4.96 ± 0.06	4.62 ± 0.05[a]	75.2 ± 2.1	72.5 ± 2.6
Rolipram (0.1 μM)	5.00 ± 0.10	4.80 ± 0.10	79.0 ± 2.1	77.6 ± 3.4
Rolipram (10 μM)	5.04 ± 0.13	4.63 ± 0.08[b]	81.3 ± 0.4	79.1 ± 0.8
Theophylline (10 μM)	5.04 ± 0.08	5.08 ± 0.07	80.7 ± 2.6	81.1 ± 2.2
Theophylline (1 mM)	4.96 ± 0.13	4.80 ± 0.12	76.6 ± 2.6	76.3 ± 2.9

The values represent the mean ± S.E.M. from five to six separate preparations. pEC_{50} and E_{max} indicate the negative logarithm of half-maximum effective concentration of histamine and maximal responses produced by histamine, respectively. E_{max} values were expressed as percentages of the contraction induced by addition of carbachol (30 μM) at end of experiments.

[a] $P < 0.01$ vs. corresponding control values (unpaired t-test).

[b] $P < 0.05$ vs. corresponding control values (unpaired t-test).

3.4. Time-courses for YM976-, rolipram- and theophylline-induced cAMP accumulation

Fig. 5 indicates time-courses for YM976 (10 μM)-, rolipram (10 μM)- and theophylline (1 mM)-induced accumulation of cAMP in the bovine tracheal preparations. These phosphodiesterase inhibitors caused time-dependent increases in cAMP accumulation. The cAMP accumulation due to each phosphodiesterase inhibitor reached a steady-state level at 5–15 min after its addition. Therefore, to examine the concentration-dependencies of YM976, rolipram and theophylline to increase the tissue cAMP content, they were assessed 15 min after addition of each agent.

3.5. Concentration-dependencies of YM976, rolipram and theophylline on cAMP accumulation

Fig. 6 shows concentration-dependencies of YM976, rolipram and theophylline on cAMP accumulation. YM976 was less potent than rolipram in increasing cAMP content at concentrations 1 μM and below. On the other

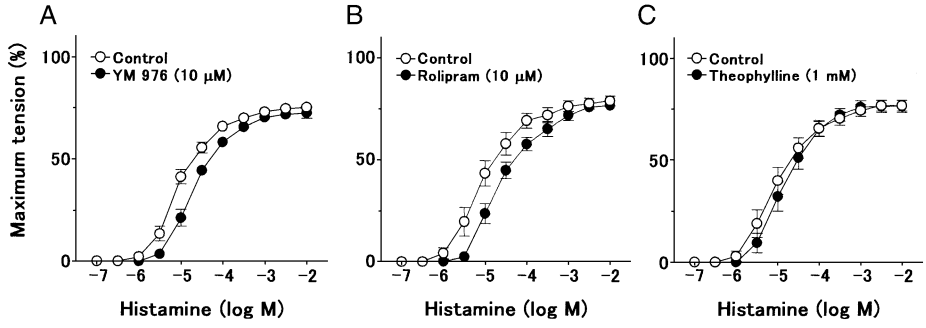

Fig. 4. Preventive effects of YM976 (10 μM), rolipram (10 μM) and theophylline (1 mM) on histamine-induced contractions in bovine tracheal smooth muscle preparations. Data are normalized as a percentage of the contraction induced by the addition of 30 μM carbachol at the end of the experiment. Each point with a vertical bar represents the mean ± S.E.M. from five to six separate preparations.

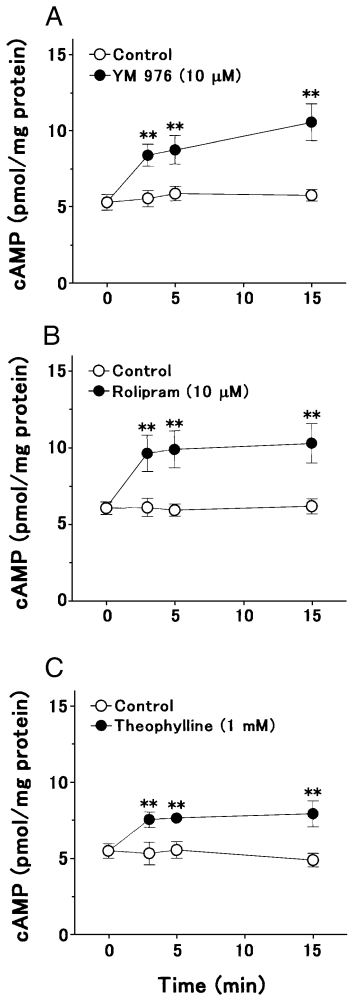

Fig. 5. Time-courses of cAMP responses of bovine tracheal smooth muscle preparations to YM976 (10 μM), rolipram (10 μM) and theophylline (1 mM). Each point with a vertical bar represents the mean ± S.E.M. from five to six separate preparations. $^{**}P<0.01$ vs. corresponding time 0 values.

hand, at 10 μM, both YM976 and rolipram exhibited almost equal accumulation of cAMP. Theophylline did not cause a significant cAMP accumulation at the concentration range below 1 mM.

4. Discussion

In the present study, we have shown that YM976, a novel and selective inhibitor of phosphodiesterase 4, markedly attenuated contractions of bovine tracheal smooth muscle induced by histamine, which is generally considered to participate in airway contraction during asthmatic attacks. At the concentrations that relaxed histamine-induced contractions, YM976 significantly enhanced the cAMP accumulation. These results suggest that YM976 accumulates intracellular cAMP due to inhibition of phosphodiesterase 4 and thereby relaxes the tracheal smooth muscle preparations.

YM976 is a novel type of phosphodiesterase 4 inhibitor synthesized based on a lead compound found by random screening. The structure is totally different from the existing phosphodiesterase 4 inhibitors, in terms of lacking the 3-cyclopentyloxy-4-methoxyphenyl group, which is shared by rolipram and others. In vitro cell-free experiments showed that YM976 was a strong and competitive inhibitor of phosphodiesterase 4. Its inhibitory effect on phosphodiesterase 4 activity was approximately 400-fold stronger than that of rolipram (IC_{50}s; YM976, 2.2 nM vs. rolipram, 820 nM) (Aoki et al., 2000). Nevertheless, YM976 was less potent than rolipram in attenuating histamine-induced contraction. Because rolipram, like YM976, increased tissue cAMP content at concentrations that attenuated histamine-induced contractions, it is likely that rolipram relaxes the bovine tracheal smooth muscle via a cAMP-dependent mechanism. However, in the case of rolipram, the concentrations that attenuated histamine-induced contractions were lower than IC_{50} values to inhibit phosphodiesterase 4 activity (~ 1.6 μM)(Giembycz and Barnes, 1991; Shahid et al., 1991; Underwood et al., 1994). Previously, Aoki et al. (2001b) have demonstrated that the EC_{50} value for rolipram inhibition of leukotriene D_4-induced contractions (50 nM) in guinea pig tracheal preparations was lower than the IC_{50} value for phosphodiesterase 4 enzyme. Also in human monocytic cells, the effective concentrations of rolipram on prostaglandin E_2-induced cAMP accumulation and lipopolysaccharide-induced tumor necrosis factor-α production

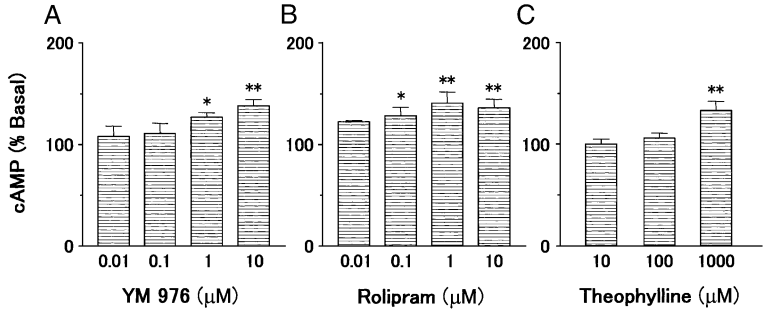

Fig. 6. Concentration-dependencies of YM976-, rolipram- and theophylline-induced cAMP accumulation in bovine tracheal smooth muscle preparations. Preparations were incubated with indicated concentration of YM976, rolipram or theophylline for 15 min, and then tissue content of cAMP was measured by radioimmunoassay. Each point with a vertical bar represents the mean ± S.E.M. from five to seven separate preparations. 100% represents cAMP accumulation in the absence of phosphodiesterase inhibitor. $^{*}P<0.05$, $^{**}P<0.01$ vs. corresponding control (CONT) values.

were lower than the IC_{50} value for phosphodiesterase 4 enzyme (Aoki et al., 2000). These results may indicate that rolipram increases the intracellular cAMP through undefined mechanisms other than inhibition of phosphodiesterase 4.

A previous study indicated that the inhibitory actions of YM976 on the precontracted guinea pig tracheal preparations and leukotriene D_4-induced bronchospasm in vivo were weaker than those of rolipram (Aoki et al., 2001b). These results were consistent with our results obtained from the current study on bovine tracheal smooth muscle. In contrast, YM976 was more potent than rolipram in preventing antigen-induced bronchoconstriction and airway hyperreactivity (Aoki et al., 2001b). YM976 has been shown to inhibit inflammatory cell functions, such as production of cytokines, superoxide formation and chemotaxis, at concentrations of nanomolar range (Aoki et al., 2000). The ability of YM976 to inhibit inflammatory cell functions was more potent than that of rolipram (Aoki et al., 2000). Thus, YM976 may suppress antigen-induced bronchoconstriction through inhibition of the spasmogen release from the leukocytes rather than by any direct relaxation effect.

YM976 was more potent than rolipram in preventing inflammatory cell functions, whereas this compound was less potent than rolipram in relaxing tracheal smooth muscle. This reason is unknown, however, two possible mechanisms could be ruled out. First, phosphodiesterase 4 has at least four subtypes (A, B, C and D), which are differently regulated and expressed in different cells (Muller et al., 1996). Although the correlation between cell function and subtype was proposed, the obvious differences in subtype selectivity between YM976 and rolipram were not found (Aoki et al., 2001a). Second, it was reported that phosphodiesterase 4 has two different conformational states, which can be distinguished by their different affinity for rolipram (i.e., the protein conformation with low affinity for rolipram and the protein conformation with high affinity for rolipram) (Souness and Rao, 1997). Recent studies suggest that the interaction of phosphodiesterase 4 inhibitors with the protein conformation with low affinity for rolipram may relate to the anti-inflammatory effects. On the other hand, the association with the protein conformation with high affinity for rolipram strongly correlates with emesis, but not with anti-inflammatory effects (Souness and Rao, 1997). However, a previous report demonstrated that YM976 exhibited the displacement of [^{3}H]rolipram with an IC_{50} value of 2.6 nM, which was almost identical to that of rolipram (1.2 nM)(Aoki et al., 2001a). Thus, neither phosphodiesterase 4 subtype selectivity nor binding for protein conformation with high affinity for rolipram explain the observation that the relaxant actions of YM976 was weaker than those of rolipram, even though YM976 was more potent than rolipram in preventing inflammatory cell functions.

The relaxant effects of phosphodiesterase inhibitors tested on preparations contracted with methacholine (0.1 μM) were weaker than those with histamine. As shown in Fig. 1, tensions developed by 0.1 μM methacholine were smaller than those by 100 μM histamine. Thus, the decreased effect of phosphodiesterase inhibitors in preparations contracted with methacholine was not due to stronger contraction. Histamine stimulates cAMP synthesis via histamine H_2 receptors that are coupled to adenylyl cyclase in the airway smooth muscle (Duncan et al., 1980; Hall et al., 1989); therefore, inhibition of phosphodiesterase may augment the accumulation of cAMP produced by histamine. However, this possibility could be ruled out, because cimetidine, a blocker of histamine H_2 receptors, did not affect the relaxant effects of YM976 and rolipram on histamine-induced contractions. At present, the mechanism by which these phosphodiesterase inhibitors are more effective against contractions produced by histamine than against those induced by methacholine is not clear, although similar observations have been shown in many previous reports (Shahid et al., 1991; Torphy et al., 1988; Heaslip et al., 1994; Raeburn et al., 1994; Fujii et al., 1998).

Some investigators demonstrated that rolipram apparently relaxed the bovine tracheal smooth muscle contracted with muscarinic agonists (Hall et al., 1990; Shahid et al., 1991; Hall and Hill, 1992). On the other hand, in this study, rolipram had only a slight relaxant action on the methacholine (0.1 μM)-contracted preparations. The reason(s) for this differential sensitivity is unknown. However, in the histamine-contracted preparations, the relaxant effect of rolipram was compatible with that reported previously (pIC_{50}, 7.1) (Shahid et al., 1991). Therefore, under our experimental conditions, the relaxant actions of phosphodiesterase inhibitors may become less marked in the preparations contracted with muscarinic agonists. Thus, even at 0.1 μM, methacholine resulted in a pronounced decrease in the ability of rolipram to relax the tracheal smooth muscle.

In this study, pretreatment with YM976 or rolipram had a significant preventive effect on the development of tensions induced by histamine. However, compared to the reversal effects, the preventive effects appear to be small. Similar phenomena have been observed in bovine (Challiss et al., 1998), guinea-pig (Bernareggi et al., 1999) and human (Fujii et al., 1998) airway smooth muscles. One possible explanation for these observations is that the mechanisms, which mediate tension generation, differ from those that maintain tension and phosphodiesterase inhibitors differentially affect these processes.

YM976 at concentrations below 1 μM was less than rolipram in histamine-contracted bovine tracheal smooth muscle; however, the higher concentrations (3 and 10 μM) of YM976 and rolipram caused the almost equal relaxations. In addition, at 10 μM YM976 and rolipram equally shifted the concentration–response curves for histamine-induced contractions to the right. Moreover, accumulation of cAMP elicited by 10 μM YM976 was similar to that by 10 μM rolipram. These results indicated that the difference between the ability of YM976 and rolipram to reverse contraction of

tracheal smooth muscle disappeared as the concentrations of both agents were increased. In other words, YM976 showed a considerable potent relaxant action on tracheal smooth muscle. Furthermore, the anti-inflammatory activities of YM976 are apparently more dissociated from its emetogenicity than other phosphodiesterase 4 inhibitors such as rolipram. Thus, YM976 would be a candidate for inflammatory diseases such as bronchial asthma.

In summary, a novel and selective inhibitor of phosphodiesterase 4, YM976, reversed histamine-induced contractions of bovine tracheal smooth muscle. YM976 also prevented the development of tensions induced by histamine. These effects elicited by YM976 in bovine tracheal smooth muscle may be related to its ability to selectively inhibit phosphodiesterase 4 and increase the cAMP content.

References

Aoki, M., Kobayashi, M., Ishikawa, J., Saita, Y., Terai, Y., Takayama, K., Miyata, K., Yamada, T., 2000. A novel phoshodiesterase type 4 inhibitor, YM976 (4-(3-chlorophenyl)-1,7,-diethypyrido[2,3-*d*]pyrimidin-2(1*H*)-one), with little emetogenic activity. J. Pharmacol. Exp. Ther. 295, 255–260.

Aoki, M., Fukunaga, M., Sugimoto, T., Hirano, Y., Kobayashi, M., Honda, K., Yamada, T., 2001a. Studies on mechanisms of low emetogenicity of YM976, a novel phosphodiesterase type 4 inhibitor. J. Pharmacol. Exp. Ther. 298, 1142–1149.

Aoki, M., Yamamoto, S., Kobayashi, M., Ohga, K., Kanoh, H., Miyata, K., Honda, K., Yamada, T., 2001b. Antiasthmatic effect of YM976, a novel PDE4 inhibitor, in guinea pigs. J. Pharmacol. Exp. Ther. 297, 165–173.

Beavo, J.A., 1995. Cyclic nucleotide phosphodiesterases: functional implications of multiple isoforms. Physiol. Rev. 75, 725–748.

Bernareggi, M.M., Belvisi, M.G., Patel, H., Barnes, P.J., Giembycz, M.A., 1999. Anti-spasmogenic activity of isoenzyme-selective phosphodiesterase inhibitors in guinea-pig trachealis. Br. J. Pharmacol. 128, 327–336.

Challiss, R.A.J., Adams, D., Mistry, R., Nicholson, C.D., 1998. Modulation of spasmogen-stimulated Ins(1,4,5)P_3 generation and functional responses by selective inhibitors of types 3 and 4 phosphodiesterase in airways smooth muscle. Br. J. Pharmacol. 124, 47–54.

Dent, G., Giembycz, M.A., Rabe, K.F., Barnes, P.J., 1991. Inhibition of eosinophil cyclic nucleotide PDE activity and opsonised zymosan-stimulated respiratory burst by "type IV-selective PDE inhibitors". Br. J. Pharmacol. 103, 1339–1346.

Duncan, P.G., Brink, C., Adolphson, R.L., Douglas, J.S., 1980. Cyclic nucleotides and contraction/relaxation in airway muscle: H_1 and H_2 agonists and antagonists. J. Pharmacol. Exp. Ther. 215, 434–442.

Fawcett, L., Baxendale, R., Stacey, P., McGrouther, C., Harrow, I., Soderling, S., Hetman, J., Beavo, J.A., Phillips, S.C., 2000. Molecular cloning and characterization of a distinct human phosphodiesterase gene family: PDE11A. Proc. Natl. Acad. Sci. U. S. A. 97, 3702–3707.

Fisher, D.A., Smith, J.F., Pillar, J.S., St. Denis, S.H., Cheng, J.B., 1998a. Isolation and characterization of PDE9A, a novel human cGMP-specific phosphodiesterase. J. Biol. Chem. 273, 15559–15564.

Fisher, D.A., Smith, J.F., Pillar, J.S., St. Denis, S.H., Cheng, J.B., 1998b. Isolation and characterization of PDE8A, a novel human cAMP-specific phosphodiesterase. Biochem. Biophys. Res. Commun. 246, 570–577.

Fujii, K., Kohrogi, H., Iwagoe, H., Hamamoto, J., Hirata, N., Goto, E., Kawano, O., Wada, K., Yamagata, S., Ando, M., 1998. Novel phosphodiesterase 4 inhibitor T-440 reverses and prevents human bronchial contraction induced by allergen. J. Pharmacol. Exp. Ther. 284, 162–169.

Fujishige, K., Kotera, J., Michibata, H., Yuasa, K., Takebayashi, S., Okumura, K., Omori, K., 1999. Cloning and characterization of a novel human phosphodiesterase that hydrolyzes both cAMP and cGMP (PDE10A). J. Biol. Chem. 274, 18438–18445.

Germain, N., Bertin, B., Legendre, A., Martin, B., Lagente, V., Payne, A., Boichot, E., 1998. Selective phosphodiesterase inhibitors modulate the activity of alveolar macrophages from sensitized guinea-pigs. Eur. Respir. J. 12, 1334–1339.

Goncalves de Moraes, V.L., Singer, M., Vargaftig, B.B., Chignard, M., 1998. Effects of rolipram on cyclic AMP levels in alveolar macrophages and lipopolysaccharide-induced inflammation in mouse lung. Br. J. Pharmacol. 123, 631–636.

Giembycz, M.A., Barnes, P.J., 1991. Selective inhibition of a high affinity type IV cyclic AMP phosphodiesterase in bovine trachealis by AH 21-132. Relevance to the spasmolytic and anti-spasmogenic actions of AH 21-132 in the intact tissue. Biochem. Pharmacol. 42, 663–677.

Hall, I.P., Hill, S.J., 1992. Effects of isozyme selective phosphodiesterase inhibitors on bovine tracheal smooth muscle tone. Biochem. Pharmacol. 43, 15–17.

Hall, I.P., Donaldson, J., Hill, S.J., 1989. Inhibition of histamine-stimulated inositol phospholipid hydrolysis by agents which increase cyclic AMP levels in bovine tracheal smooth muscle. Br. J. Pharmacol. 97, 603–613.

Hall, I.P., Donaldson, J., Hill, S.J., 1990. Modulation of carbachol-induced inositol phosphate formation in bovine tracheal smooth muscle by cyclic AMP phosphodiesterase inhibitors. Biochem. Pharmacol. 39, 1357–1363.

Harris, A.L., Connel, M.J., Ferguson, E.W., Wallace, A.M., Gordon, R.J., Pagani, E.D., Silver, P.J., 1989. Role of low Km cyclic AMP phosphodiesterase inhibition in tracheal relaxation and bronchodilation in the guinea pig. J. Pharmacol. Exp. Ther. 251, 199–206.

Hayashi, M., Matsushima, K., Ohashi, H., Tsunoda, H., Murase, S., Kawarada, Y., Tanaka, T., 1998. Molecular cloning and characterization of human PDE8B, a novel thyroid-specific isozyme of 3′,5′-cyclic nucleotide phosphodiesterase. Biochem. Biophys. Res. Commun. 250, 751–756.

Heaslip, R.J., Lombardo, L.J., Golankiewicz, J.M., Ilsemann, B.A., Evans, D.Y., Sickels, B.D., Mudrick, J.K., Bagli, J., Weichman, B.M., 1994. Phosphodiesterase-IV inhibition, respiratory muscle relaxation and bronchodilation by WAY-PDA-641. J. Pharmacol. Exp. Ther. 268, 888–896.

Hetman, J.M., Soderling, S.H., Glavas, N.A., Beavo, J.A., 2000. Cloning and characterization of PDE7B, a cAMP-specific phosphodiesterase. Proc. Natl. Acad. Sci. U. S. A. 97, 472–476.

Holbrook, M., Gozzard, N., James, T., Higgs, G., Hughes, B., 1996. Inhibition of bronchospasm and ozone-induced airway hyperresponsiveness in the guinea-pig by CDP840, a novel phosphodiesterase type 4 inhibitor. Br. J. Pharmacol. 118, 1192–1200.

Katsuki, S., Murad, F., 1977. Regulation of adenosine cyclic 3′,5′-monophosphate and guanosine cyclic 3′,5′-monophosphate levels and contractility in bovine tracheal smooth muscle. Mol. Pharmacol. 13, 330–341.

Loughney, K., Snyder, P.B., Uher, L., Rosman, G.J., Ferguson, K., Florio, V.A., 1999. Isolation and characterization of PDE10A, a novel human 3′,5′-cyclic nucleotide phosphodiesterase. Gene 234, 109–117.

Muller, T., Engels, P., Fozard, J.R., 1996. Subtypes of the type 4 cAMP phosphodiesterase: structure, regulation and selective inhibition. Trends Pharmacol. Sci. 17, 294–298.

Peachell, P.T., Undem, B.J., Schleimer, R.P., MacGlashan Jr., D.W., Lichtenstein, L.M., Cieslinski, L.B., Torphy, T.J., 1992. Preliminary identification and role of phosphodiesterase isozymes in human. J. Immunol. 148, 2503–2510.

Raeburn, D., Underwood, S.L., Lewis, S.A., Woodman, V.R., Battram, C.H., Tomkinson, A., Sharma, S., Jordan, R., Souness, J.E., Webber, S.E., Karlsson, J.-A., 1994. Anti-inflammatory and bronchodilator properties of RP 73401, a novel and selective phosphodiesterase type IV inhibitor. Br. J. Pharmacol. 113, 1423–1431.

Shahid, M., van Amsterdam, R.G.M., de Boer, J., Ten Berge, R.E., Nicholson, C.D., Zaagsma, J., 1991. The presence of five cyclic nucleotide phosphodiesterase isoenzymes activities in bovine tracheal smooth

muscle and the functional effects of selective inhibitors. Br. J. Pharmacol. 104, 471–477.

Soderling, S.H., Bayuga, S.J., Beavo, J.A., 1999. Isolation and characterization of a dual-substrate phosphodiesterase gene family: PDE10A. Proc. Natl. Acad. Sci. U. S. A. 96, 7071–7076.

Souness, J.E., Rao, S., 1997. Proposal for pharmacologically distinct conformers of PDE4 cyclic AMP phosphodiesterases. Cell Signal 9, 227–236.

Torphy, T.J., Burman, M., Huang, L.B.F., Tucker, S.S., 1988. Inhibition of the low Km cyclic AMP phosphodiesterase in intact canine trachealis by SK&F 94836: mechanical and biochemical responses. J. Pharmacol. Exp. Ther. 246, 843–850.

Torphy, T.J., Barnette, M.S., Underwood, D.C., Griswold, D.E., Christensen, S.B., Murdoch, R.D., Nieman, R.B., Compton, C.H., 1999. Ariflo (SB 207499), a second generation phosphodiesterase 4 inhibitor for the treatment of asthma and COPD: from concept to clinic. Pulm. Pharmacol. Ther. 12, 131–135.

Underwood, D.C., Osborn, R.R., Novak, L.B., Matthews, J.K., Newsholme, S.J., Undem, B.J., Hand, J.M., Torphy, T.J., 1993. Inhibition of antigen-induced bronchoconstriction and eosinophil infiltration in the guinea pig by the cyclic AMP-specific phosphodiesterase inhibitor, rolipram. J. Pharmcaol. Exp. Ther. 266, 306–313.

Underwood, D.C., Kotzer, C.J., Bochnowicz, S., Osborn, R.R., Luttmann, M.A., Hay, D.W.P., Torphy, T.J., 1994. Comparison of phosphodiesterase III, IV and dual III/IV inhibitors on bronchospasm and pulmonary eosinophil influx in guinea pigs. J. Pharmcaol. Exp. Ther. 270, 250–259.

Weston, M.C., Anderson, N., Peachell, P.T., 1997. Effects of phosphodiesterase inhibitors on human lung mast cell and basophil function. Br. J. Pharmacol. 121, 287–295.