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Leukotriene Receptor Antagonism and Augmentation of β-Receptor-mediated Events by LY171883[1]

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Abstract—LY171883, (1-[2-hydroxy-3-propyl-4-((4(1H-tetrazol-5-yl)butoxy)phenyl]ethanone), a leukotriene (LT) D_4/E_4 receptor antagonist, was assessed in comparison with two well known phosphodiesterase inhibitors, isobutylmethyl-xanthine (IBMX) and theophylline, for its ability to augment β-receptor-mediated responses. Relaxation of carbachol-contracted guinea-pig trachea by isoprenaline was enhanced by the three agents in a dose-dependent manner. A two-fold enhancement of isoprenaline-induced smooth muscle relaxation was produced by 2·5 μM IBMX, 28 μM LY171883, or 140 μM theophylline. Similar concentrations of IBMX or theophylline did not antagonize LTE_4-induced tracheal contractions; LY171883 totally inhibited the response and had significant LTE_4 receptor antagonist activity even at 10-fold lower concentrations. Antigen-induced release of histamine and LTC_4 from guinea-pig lung was reduced by isoprenaline. Prior treatment with LY171883, IBMX, or theophylline did not enhance this action. Isoprenaline reduced histamine-induced bronchospasm in anaesthetized guinea-pigs. LY171883, 30 mg kg^{-1}, or IBMX, 1 mg kg^{-1}, did not affect the isoprenaline-induced decrease in the histamine response. IBMX, 3 mg kg^{-1}, and theophylline, 30 mg kg^{-1}, augmented the isoprenaline-induced bronchodilation. LTE_4-induced bronchoconstriction was not affected by IBMX or theophylline whereas LY171883 antagonized this response at doses as low as 3 mg kg^{-1}. Therefore, in both in-vitro and in-vivo test systems, LY171883 functioned primarily as a leukotriene receptor antagonist with minimal pharmacological activity attributable to its ability to potentiate isoprenaline.

The cysteinyl leukotrienes, LTC_4, LTD_4, and LTE_4, constrict airway smooth muscle (Drazen 1986; Samuelsson et al 1987), increase mucus production (Kaliner et al 1984), and decrease mucociliary transport (Russi et al 1985). Because of these actions, many LTD_4/LTE_4 receptor antagonists are currently in development for the treatment of asthma. LY171883 (1-[2-hydroxy-3-propyl-4-((4(1H-tetrazol-5-yl)butoxy)phenyl] ethanone), was one of the first LTD_4/LTE_4 receptor antagonists tested in human asthmatics. This compound blocks contractions of guinea-pig isolated smooth muscle to LTD_4 and LTE_4, but not to LTC_4, LTB_4, prostaglandin $F_{2\alpha}$, 5-HT, carbamylcholine, histamine, or bradykinin. Tracheal contractions to U46619, an agonist at thromboxane A_2 receptors, were slightly reduced by LY171883 (Fleisch et al 1985). It was also active as a leukotriene receptor antagonist after oral administration to animals (Fleisch et al 1985) or man (Phillips et al 1988).

Cloud et al (1989) recently reported that LY171883-treated mild-to-moderate asthmatics showed greater improvement in their FEV_1 values during the six week treatment, than a placebo-treated group. All patients were permitted orciprenaline (metaproterenol) metered-dose inhalers as required to reduce unresolved bronchospasm. Patients taking small doses of orciprenaline per week to control their symptoms, used similar amounts at the beginning and end of the treatment. Individuals who required more than 23 mg of orciprenaline per week substantially reduced their use of this β-adrenoceptor agonist.

In addition to leukotriene receptor antagonism, LY171883 and other members of the acetophenone chemical class inhibit phosphodiesterase (Chasin & Scott 1978; Fleisch et al 1985, 1986) an enzyme that catalyses the breakdown of cAMP to 5′-AMP (Rall 1982). Inhibition of this enzyme can result in bronchodilation and potentiation of β-adrenoceptor agonists (Armour et al 1982; Lorenz & Wells 1983; Schoeffter et al 1987; Taylor 1987), which might have contributed to the clinical efficacy of LY171883. To address this issue, the relative ability of LY171883 to antagonize LTE_4 has been compared with its capacity to augment β-adrenoceptor activity. IBMX and theophylline, two well-characterized phosphodiesterase inhibitors (Korth 1978; Weishaar et al 1985), were tested along with LY171883.

Materials and Methods

In-vitro experiments

Guinea-pig trachea. Male Hartley guinea-pigs (Charles River Breeding Laboratories, Portage, Michigan), 300–400 g, were decapitated and tracheas excised, cleaned of connective tissue, cut into four ring segments, and placed on stainless steel supports. Tissues were suspended in 10 mL organ baths containing Krebs bicarbonate solution, composition in mM: KCl, 4·6; KH_2PO_4, 1·2; $MgSO_4 \cdot 7H_2O$, 1·2; NaCl, 118·2; $NaHCO_3$, 24·8; dextrose, 10·0 and $CaCl_2 \cdot 2H_2O$, 2·5. Indomethacin, 3×10^{-6} M, was incorporated into the bathing solution to prevent production of prostaglandins that might influence contractile responses. The temperature was maintained at 37°C and the bathing solution aerated with 95% O_2

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and 5% CO_2. Tracheas were equilibrated for 1 h, resting tension was 2 g. Isometric measurements were made with Grass FT03C force-displacement transducers and recorded on a Grass Model 7 polygraph as changes in grams of force.

Potentiation of tracheal responses to isoprenaline. Following equilibration, tracheas were contracted three times with 2×10^{-7} M carbachol. This concentration produced a response that was 60–80% of maximum. The third carbachol-induced contraction required approximately 30 min to attain a steady state condition and this contraction was well maintained in those tissues not relaxed with isoprenaline. When steady state was reached, isoprenaline was added to the bath in a cumulative manner to generate a relaxation concentration-response curve. Additions of isoprenaline were at 5–15 min intervals and made when the induced relaxation had reached a plateau. The concentration that produced a 50% tracheal relaxation was determined using linear regression. The tissues were washed and vehicle, LY171883, IBMX, or theophylline was added to the baths. Thirty minutes later, the tracheas were contracted again with carbachol and relaxed with isoprenaline.

Leukotriene E_4 receptor antagonism. Tracheas were contracted three times with 1×10^{-5} M histamine. The tissues were washed and, following return to baseline, vehicle, LY171883, IBMX, or theophylline was added to each bath. At the end of the 30 min incubation, cumulative concentration-response curves were run to LTE_4. Only one concentration-response curve was run on each tissue; one tissue from each set of four served as control for the other segments.

Mediator release experiments. Male Hartley guinea-pigs, 7–10 days of age, were killed by CO_2 asphyxiation. Lungs were excised and perfused through the pulmonary artery with Krebs bicarbonate solution containing 1·8 mM $CaCl_2$. Poorly perfused areas were discarded. Normal lung was cut into 1 mm cubes with a McIlwain tissue chopper, washed with Krebs bicarbonate solution, divided into 400 mg samples, and incubated for 1 h at 37 C in vials containing 2·5 mL of hyperimmune serum diluted 1/25 with Krebs bicarbonate buffer. Hyperimmune serum was previously prepared by actively sensitizing guinea-pigs with 2 mg ovalbumin in 50% Complete Freunds' adjuvant i.p. on days 1 and 5. On day 21 the animals were bled and serum collected.

After passive sensitization, the tissues were washed with Krebs bicarbonate solution containing 1×10^{-6} M indomethacin, to optimize leukotriene release, 1×10^{-6} M phentolamine, to minimize the α-adrenoreceptor actions of isoprenaline, and 1×10^{-7} M ascorbic acid, to prevent oxidation of isoprenaline. Tissue samples were incubated at 37 C for 20 min in 2·0 mL of Krebs bicarbonate solution containing appropriate amounts of drug or vehicle. Isoprenaline (250 μL, 1×10^{-8} M) or vehicle was added to vials and incubation continued for 10 min. Antigen (250 μL, 3×10^{-4} g mL^{-1}) was added to give final concentrations of 3×10^{-5} g mL^{-1} for ovalbumin and 1×10^{-9} M for isoprenaline, and the incubation continued for an additional 15 min. The medium was then decanted into tubes. Histamine remaining after antigen challenge was released from the tissue by addition of 2·5 mL of Krebs bicarbonate solution to the vials and immersing them in boiling water for 10 min. The medium was decanted and all supernatants were centrifuged at 3000 *g* for 5 min. Histamine content of the samples was determined using the enzymatic method of Verberg & Henry (1986). The amount of histamine released into the incubation medium by boiling, plus that released by antigen challenge was considered the total histamine content of the tissue. Antigen released approximately 29% of total tissue histamine. Supernatants from guinea-pig chopped lung were also analysed for $iLTC_4$ (immunoreactive LTC_4) using the radioimmunoassay described by Evers et al (1985). The cross reactivities of the LTC_4 antisera were 50% toward LTD_4, 8% toward LTE_4 and less than 0·01% toward LTB_4, thromboxane B_2, prostaglandins E_2, $F_{2\alpha}$, A_2, and D_2, 5-HETE, 9-HETE, 11-HETE, arachidonic acid, and glutathione.

In-vivo experiments

Measurement of intratracheal pressure. Male Hartley guinea-pigs, 300–400 g, were anaesthetized with 45–50 mg kg^{-1} sodium pentobarbitone i.p. Both jugular veins were cannulated with polyethylene catheters (PE 50) for administering drugs. The trachea was also cannulated (PE 240) and the animal ventilated with room air using a Harvard rodent respirator set to deliver a tidal volume of 1 mL/100 grams body weight 50 times min^{-1}. Succinylcholine, 5 mg kg^{-1}, was given intravenously to suppress spontaneous respiration. Intratracheal pressure, a reflection of bronchomotor tone, was measured with a Statham pressure transducer (P23ID) connected to a T-tube on the tracheal cannula. This procedure is a modification of the Konzett-Rossler technique (1940). Output signals from the pressure transducers were recorded on a Grass model 79D polygraph. Body temperature was maintained within normal limits by means of a Deltaphase Isothermal Pad (Braintree Scientific, Inc, Braintree, MA, USA). At the end of each experiment, the trachea was clamped off and maximal pressure determined. Intratracheal pressure was quantitated as a percentage of this maximum.

Potentiation of isoprenaline-induced bronchodilation. Ten minutes after succinylcholine, a histamine dose-response curve was obtained by giving 3–5 different doses of histamine i.v. at 3 min intervals and determining the degree of bronchoconstriction. Linear regression was used to determine the ED50. Isoprenaline was infused at a constant rate, 1–5 μg min^{-1}, and another dose response curve to histamine obtained. Animals were randomized, LY171883, IBMX, theophylline, or vehicle was administered and 20 min later the histamine dose-response curve was repeated followed by isoprenaline infusion and a final histamine dose-response curve. Thus, four histamine dose-response curves were determined in each animal. Dose ratios for the isoprenaline-induced shift in the histamine dose–response curves were determined by dividing the histamine ED50 during isoprenaline by the histamine ED50 before isoprenaline. The influence of LY171883, IBMX, or theophylline on the bronchodilator action of isoprenaline was determined by comparing the isoprenaline-induced dose ratios generated before and after drug administration. Statistical differences in the before and after drug dose ratios were determined using an analysis of variance and Fisher's protected least-

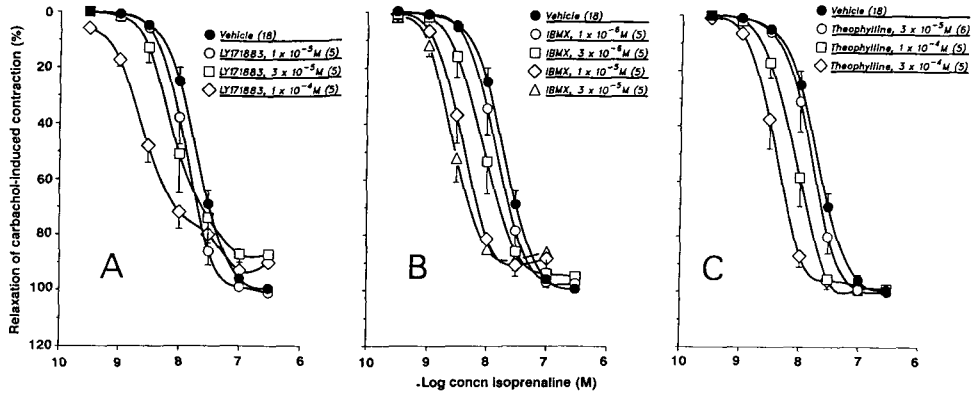

FIG. 1. LY171883 (A), IBMX (B) or theophylline (C) produced concentration dependent potentiation of isoprenaline-induced tracheal relaxation. Values are means ± standard error of the mean for the number of tissues shown in the key.

significant difference procedure. The paired nature of the data was taken into account by subtracting the before drug dose ratio from the after drug dose ratio for each individual experiment. Dose ratios were log transformed to eliminate skewness.

LTE_4 receptor antagonism. Two minutes after succinylcholine, propranolol, a β-adrenoceptor antagonist, was administered to the guinea-pigs in a dose of 1 mg kg^{-1}. After an additional 10 min, 3–5 doses of LTE_4 were given in ascending order at 3 min intervals. Five min after the last dose of LTE_4, LY171883, IBMX, theophylline, or vehicle was administered. Fifteen min later, a second LTE_4 dose-response curve was obtained.

Materials

Drugs used were: carbachol (carbamylcholine chloride), histamine dihydrochloride, indomethacin, succinylcholine chloride, isoprenaline (−)-bitartrate, (±)-propranolol hydrochloride (Sigma Chemical Co., St Louis, MO), sodium pentobarbitone (Butler Co., Columbus, OH), 3-isobutyl-1-methylxanthine (Aldrich Chemical Co., Milwaukee, WI), theophylline, ascorbic acid, LY171883, and LTE_4 (Eli Lilly and Co. Indianapolis, IN and Windlesham, UK).

Results

Carbachol-contracted guinea-pig tracheas were relaxed by isoprenaline. LY171883, IBMX, or theophylline, enhanced this response in a dose-dependent manner (Fig. 1). The concentrations causing a two-fold leftward shift of the isoprenaline concentration-response curve were $2{\cdot}5 \times 10^{-6}$ M IBMX, $2{\cdot}8 \times 10^{-5}$ M LY171883, and $1{\cdot}4 \times 10^{-4}$ M theophylline. Thus, IBMX was about 10 times more potent than LY171883, which in turn was five times more potent than theophylline.

The relationship of this potentiation of β-receptor-mediated tracheal relaxation to LTE_4 receptor antagonism was investigated in the next group of experiments. LTE_4-induced contractions of guinea-pig trachea were obtained in the presence and absence of those concentrations of LY171883, IBMX, or theophylline which produced two-fold potentiation of isoprenaline. IBMX and theophylline did not

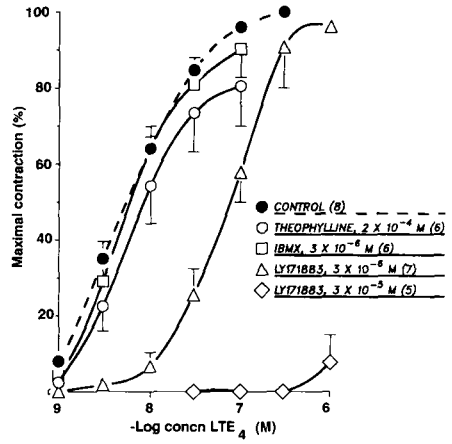

FIG. 2. At concentrations that caused 2-fold potentiation of isoprenaline-induced tracheal relaxation, IBMX and theophylline were not LTE_4 receptor antagonists, whereas LY171883 markedly antagonized LTE_4 even at a 10-fold lower concentration.

antagonize tracheal contractions elicited by the leukotriene (Fig. 2). In contrast, LY171883, at a 10-fold lower concentration, caused a marked displacement of the LTE_4 concentration-response curve. At 3×10^{-5} M, contractions to LTE_4 were essentially abolished (Fig. 2).

Complementary in-vivo experiments were performed in anaesthetized guinea-pigs. Histamine, given i.v., produced a dose-related bronchoconstriction with an ED50 of 12 μg kg^{-1}. Infusion of isoprenaline, 1–5 μg min^{-1}, caused a four-fold shift to the right of the histamine dose-response curve (Table 1). Neither 30 mg kg^{-1} LY171883 nor 1 mg kg^{-1} of IBMX affected this action of isoprenaline. However, both theophylline, 30 mg kg^{-1}, and IBMX, 3 mg kg^{-1}, enhanced the ability of isoprenaline to counteract the histamine-induced bronchoconstriction (Table 1).

LY171883, IBMX, and theophylline were then evaluated as LTE_4 receptor antagonists in a separate group of guinea-pigs. LY171883 proved to be an effective antagonist of LTE_4 at doses which did not potentiate isoprenaline (Fig. 3). Neither IBMX nor theophylline reduced the increase in intratracheal pressure elicited by LTE_4 when administered at doses that potentiated isoprenaline.

Table 1. Effects of LY171883, IBMX or theophylline on isoprenaline-induced bronchodilation.

	Isoprenaline dose-ratios[a]	
Drug	Before drug	After drug
Vehicle (7)[b]	4·49 ± 0·98	4·23 ± 1·22
LY171883, i.v.		
30 mg kg^{-1} (8)	5·31 ± 1·37	6·48 ± 2·57
Theophylline, i.v.		
30 mg kg^{-1} (9)	4·79 ± 1·53	6·59 ± 1·36*
IBMX, i.v.		
1 mg kg^{-1} (8)	4·12 ± 0·74	5·46 ± 1·21
3 mg kg^{-1} (8)	4·39 ± 1·11	8·78 ± 2·44**

[a] Values shown are means and s.e.m.s of the dose ratio induced by isoprenaline: μg kg^{-1} histamine which produced 50% of the maximum responses (ED50) during isoprenaline infusion divided by the ED50 before isoprenaline infusion.
[b] Number of animals.
Significant difference from vehicle: * $P < 0.05$, ** $P < 0.001$.

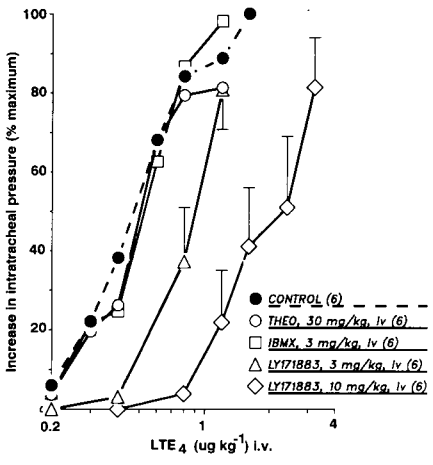

FIG. 3. LTE_4-induced bronchoconstriction was not antagonized by doses of IBMX or theophylline which potentiated isoprenaline. Dose dependent antagonism of LTE_4-induced bronchospasm was observed at 3 and 10 mg kg^{-1} LY171883. LY171883, 30 mg kg^{-1}, which had no effect on responses to isoprenaline, abolished the LTE_4-induced bronchoconstriction (not shown).

Isoprenaline reduces the quantity of histamine and leukotrienes released from immunologically sensitized lung in response to antigen challenge (Austen 1974; Fleisch et al 1979). To determine if LY171883, IBMX, or theophylline would enhance this action, sensitized guinea-pig lung samples were incubated with each of the three agents alone and in combination with isoprenaline. Samples were challenged with antigen; histamine and $iLTC_4$ levels were measured. Isoprenaline alone, 1×10^{-9} M, inhibited antigen-induced histamine release by 40% and reduced $iLTC_4$ release by 37% (Table 2). IBMX, theophylline, and LY171883 had a variable effect on histamine release as judged by the large standard errors, but did not significantly inhibit release. Inhibition of $iLTC_4$ release was more marked. Theophylline and LY171883 significantly reduced leukotriene release at the concentrations tested. However, none of these agents amplified the action of isoprenaline. The sum of mediator release inhibition by isoprenaline plus that attributed to the individual test drugs was similar in magnitude to the inhibition of mediator release caused by their combination (Table 2). This suggests that their effects were additive and not synergistic.

Discussion

Cysteinyl leukotrienes have been proposed to mediate some of the symptoms associated with human asthma (Austen & Soberman 1988; Barnes et al 1988). Considerable effort has been expended to develop selective drugs that might reduce the severity or completely abolish the clinical expression of this disorder (Perchonock et al 1987; Fleisch et al 1988a,b). LY171883 (Fleisch et al 1985), although no longer undergoing clinical evaluation, has been invaluable to future development of leukotriene receptor antagonists. An unanswered question regarding LY171883 was how significantly its ability to augment β-receptor activity contributed to its modest clinical efficacy. This was especially relevant to the study by Cloud et al (1989) in which a group of asthmatics reduced their usage of orciprenaline, a β-adrenoceptor agonist, during administration of LY171883.

Inhibition of phosphodiesterase amplifies the action of β-receptor agonists (Armour et al 1982; Lorenz & Wells 1983; Schoeffter et al 1987; Taylor 1987). This enzyme exists as a family of isozymes which can be selectively inhibited by various drugs (Bergstrand & Lundquist 1978; Weishaar et al 1985; Silver et al 1988). Thus, a drug might be a potent inhibitor of one form of phosphodiesterase yet exert little or no activity against an isozyme associated with β-receptor activity. LY171883 is a relatively weak phosphodiesterase inhibitor (Fleisch et al 1985). In the present investigation, we designed pharmacological studies to compare the potential consequences of phosphodiesterase inhibition and of leukotriene receptor antagonism in the same biological systems. The results presented here reflect inhibition only of the most relevant isozymes.

Isoprenaline, the prototypic β-adrenoceptor agonist, relaxed carbachol-contracted guinea-pig trachea. LY171883, IBMX, and theophylline all augmented this response as expected of phosphodiesterase inhibitors. At concentrations causing a two-fold potentiation of isoprenaline, neither IBMX nor theophylline reduced tracheal contractions elicited by LTE_4. In sharp contrast, LY171883 virtually abolished the response to LTE_4. Furthermore, even a 10-fold lower concentration, 3×10^{-6} M, produced a marked rightward shift of the LTE_4 concentration-response curve. From these results, we conclude that, unlike IBMX or theophylline, LY171883 is a leukotriene receptor antagonist at concentrations well below those that potentiate isoprenaline. Thus, in this in-vitro setting, there was a clear separation of these two distinct pharmacological activities. A separation of leukotriene receptor antagonism and β-receptor agonist potentiation was also demonstrated by Armour et al (1982). They found theophylline unable to antagonize LTD_4-induced contractions of guinea-pig trachea, although it enhanced the effect of salbutamol, a β-adrenoceptor agonist, when the two agents were combined.

Table 2. Lack of augmentation by LY171883, IBMX, or theophylline of isoprenaline-induced inhibition of mediator release from guinea-pig lung.

		% Inhibition of histamine release[a]		
			Drug + isoprenaline	
Drug	Concn (M)	Drug 2	Separately[b]	Simultaneously[c]
Isoprenaline	1×10^{-9}	40·5 ± 3·0*		
IBMX	3×10^{-6}	9·1 ± 10·9	49·6 ± 13·7	59·2 ± 8·1
Theophylline	2×10^{-4}	18·0 ± 15·0	58·5 ± 17·1	56·0 ± 2·6
LY171883	3×10^{-5}	17·1 ± 9·3	57·6 ± 7·5	53·3 ± 4·9
		% Inhibition of iLTC$_4$ release[a]		
			Drug + isoprenaline	
			Separately[b]	Simultaneously[c]
Isoprenaline	1×10^{-9}	37·4 ± 7·8*		
IBMX	3×10^{-6}	17·1 ± 10·5	54·5 ± 11·1	55·4 ± 10·6
Theophylline	2×10^{-4}	22·7 ± 7·4*	60·1 ± 12·4	57·5 ± 4·3
LY171883	3×10^{-5}	45·0 ± 8·9*	82·3 ± 11·1	65·6 ± 3·6
	3×10^{-6}	24·8 ± 4·5*	56·2 ± 10·9	40·3 ± 9·4

[a] Values are means ± standard error of the mean for 3 or 4 experiments.
[b] Sum of inhibition of mediator release caused by isoprenaline and inhibition caused by drug alone.
[c] Observed inhibition of mediator release when isoprenaline and drug were both present.
Statistically significant inhibition of mediator release: * $P < 0.05$, ** $P < 0.01$.

The in-vivo corollary of the preceding experiments was performed in anaesthetized guinea-pigs. Intravenous administration of isoprenaline blunted histamine-induced bronchoconstriction. This response to isoprenaline could be enhanced by pretreatment with theophylline and to an even greater extent by IBMX. LY171883, at doses up to 30 mg kg^{-1}, did not alter the apparent bronchodilation to isoprenaline. However, a much lower dose of LY171883, 3 mg kg^{-1}, significantly blunted the bronchoconstrictor response to LTE$_4$. Neither IBMX nor theophylline blocked a similar response to LTE$_4$. These observations indicate that a pharmacological consequence of phosphodiesterase inhibition, namely amplification of β-receptor activity, does not measurably contribute to the in-vivo activity of LY171883.

Another experimental test system relevant to asthma is represented by antigen-induced release of anaphylactic mediators. β-Adrenoceptor stimulation reduces mediator release from guinea-pig fragmented lung (Assen & Schild 1971; Sorenby 1975). At concentrations that enhanced isoprenaline-induced relaxation of guinea-pig trachea, LY171883, theophylline or IBMX did not modify the action of isoprenaline on histamine or iLTC$_4$ release. The extent of mediator release inhibition produced by the combination of either LY171883, theophylline or IBMX with isoprenaline reflected the sum of each drug separately. Studies by Wong & Buckner (1980) also demonstrated that the effects of phosphodiesterase inhibitors and isoprenaline on histamine release from antigen-challenged guinea-pig lung were additive rather than synergistic.

The present study has produced in-vitro and in-vivo evidence of a dissociation of two pharmacological characteristics of LY171883. At concentrations most likely to be used therapeutically, virtually all the pharmacology of LY171883 can be attributed to leukotriene receptor antagonism (Fleisch et al 1985; present study). Higher concentrations of LY171883 would be necessary to augment β-adrenergic receptor agonist activity.

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