# Inhibitory Effect of a Novel Phosphodiesterase IV Inhibitor, T-440, on Antigen- and Chemical Mediator-Induced Bronchoconstrictions in Guinea Pigs In Vivo

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*Received February 28, 1996 Accepted June 3, 1996*

ABSTRACT—We demonstrated the effect of a novel selective type IV phosphodiesterase (PDE) IV inhibitor, T-440 (1-[1-(2-methoxyethyl)pyrid-2-one-4-yl]-2,3-bis(hydroxymethyl)-6,7-diethoxynaphthalene), on antigen- and chemical mediator-induced bronchoconstrictions in anesthetized guinea pigs in vivo. Intravenously (i.v.) administered T-440 inhibited antigen-induced bronchoconstriction dose-dependently in passively sensitized guinea pigs ($ED_{50}$=2.3 mg/kg). Histamine-, leukotriene (LT) $D_4$-, U-46619-, acetylcholine (ACh)-, neurokinin A- and endothelin-1-induced bronchoconstrictions were also inhibited by i.v. injected T-440. Most potent suppression was produced against the bronchoconstriction induced by $LTD_4$ ($ED_{50}$=0.89 $\mu$g/kg), whereas the effect against ACh was very weak ($ED_{50}$=1.8 mg/kg). Additionally, T-440 inhibited histamine-induced bronchoconstriction by intraduodenal and intratracheal administration ($ED_{50}$ and $EC_{50}$=1.6 mg/kg and 0.50 mg/ml, respectively). Bronchoconstrictions induced by antigen and chemical mediators were also suppressed by theophylline. However, all of these anti-spasmolytic effects of theophylline were less potent than those of T-440 (1.8-110 times). Our results indicate the importance of PDE IV in bronchodilation, and PDE IV inhibitors may have potential as anti-asthma drugs.

*Keywords*: Phosphodiesterase, Airway, Asthma, Bronchoconstriction

Bronchial asthma is characterized clinically as recurrent wheezing dyspnea occurring in patients provocated with variety of antigens. This anaphylactic bronchoconstriction is mediated primarily by the actions of preformed (1) and newly generated (2, 3) chemical mediators released from mast cells that are associated with bronchial mucosa and activated by antigen-antibody reactions (4). Therefore, a drug that inhibits antigen-induced bronchoconstriction is anticipated to provide a possible way to prevent or alleviate asthmatic attack.

Xanthines, represented by theophylline, have long been used in asthma therapy because of their relaxing effect on the bronchial smooth muscle (5, 6). Nevertheless, theophylline has other multiple pharmacological activities such as central (7), cardiovascular (8, 9) and digestive actions (5) and shows a very low margin of safety (6, 10, 11). It was reported that the mechanism underlying the effect of theophylline is related to its inhibitory activity against the enzyme phosphodiesterase (PDE) and subsequent elevation of intracellular cAMP (12). Currently, at least 7 different PDE isozyme gene families are recognized in many types of cells (13, 14). The airway smooth muscle contains mainly PDE III and PDE IV, and both of the isozymes regulate airway tone, although PDE III is also related to some cardiovascular side effects (13, 14). Therefore, the side effects of theophylline seem to be caused partly by the non-selectivity for PDE inhibition. This situation prompted us to explore the actions of a selective PDE IV inhibitor that shows potent bronchodilator activity.

In the present study, we examined the effect of a selective PDE IV inhibitor, T-440 (1-[1-(2-methoxyethyl)pyrid-2-one-4-yl]-2,3-bis(hydroxymethyl)-6,7-diethoxynaphthalene), on antigen and chemical mediator-induced bronchoconstrictions in anesthetized guinea pigs in vivo. T-440 was elaborated during a search for an anti-asthma drug. We previously reported that this drug selectively inhibited the activity of PDE IV purified from the guinea pig lung (15). Now, detailed pharmacological studies revealed that T-440 displayed the inhibitory effect on the bronchoconstriction produced by antigen and chemical mediators in anesthetized guinea pigs. Furthermore, the

efficiency of T-440 was demonstrated by multiple administration routes, such as intravenous (i.v.), intraduodenal (i.d.) and intratracheal (i.t.) applications. These effects of T-440 were compared with those of theophylline.

## MATERIALS AND METHODS

*Antigen-induced bronchoconstriction in anesthetized guinea pigs*

Anti-ovalbumin (OA) rabbit antiserum was prepared from rabbits (2.0−2.5 kg; Japan KBL, Ina) that had been immunized by intramuscular injection of OA (10−20 mg) emulsified with Freund's complete adjuvant 4 times weekly. The serum was obtained 7 days after the last immunization and stored frozen below −70℃ until use. The antibody titers of the antiserum thus obtained were $>10^4$ times as determined by the 4-hr PCA reaction test in guinea pigs. Male Hartley guinea pigs (290−595 g; Japan SLC, Hamamatsu) were sensitized by i.v. administration of anti-OA rabbit antiserum (0.5 ml/kg). Twenty to 28 hr later, animals were anesthetized with $\alpha$-chloralose (120 mg/kg, i.v.) so that they could be inserted with tracheotomy tubes and the lateral saphenous vein was cannulated for i.v. injection. They were then immobilized by injection of gallamine triethiodide solution (5 mg/kg, i.v.) under the artificial respiration by a small respirator set (model 680-683; Harvard, South Natick, MA, USA) at a rate of 60 breaths per min. The changes in pulmonary mechanics were measured in accordance with the method of Konzett and Rössler (16) with a differential pressure transducer (TP-602; Nihon Kohden, Tokyo) connected to a T-tube on the tracheal cannula. The increase in ventilation overflow volume ($\Delta$VOFV) provoked by antigen challenge was expressed as a percentage of the maximum bronchoconstriction obtained by clamping off the trachea. Test compounds were administered i.v. 2 min before the antigen challenge. The effect of the test compounds was expressed as inhibition of $\Delta$VOFV induced by i.v. injection of antigen solution (30 $\mu$g/kg). The inhibitory potency of the drugs was expressed as the dose that suppressed the antigen-induced bronchoconstriction by 50% ($ED_{50}$). Heart rate was monitored by cardiotachography utilizing the R wave of ECG (standard limb lead II) as a trigger.

*Chemical mediator-induced bronchoconstriction in anesthetized guinea pigs*

Animals were anesthetized and immobilized under artificial respiration in accordance with the measurement of antigen-induced bronchoconstriction. The duodenum was cannulated when test compounds were administered i.d. For i.t. administration, an ultrasonic nebulizer (TUR-3000, Nihon Kohden) was inserted in the respiratory route between the respirator and tracheal cannula. Bronchoconstrictions induced by chemical mediators excepting endothelin (ET)-1 were expressed as the increase in pulmonary inflation pressure ($\Delta$PIP) measured with a pressure transducer (TP-200T, Nihon Kohden). When test compounds were administered i.d., histamine (2 $\mu$g/kg) was injected every 15 min and in other cases, injected with a 10-min interval. Acetylcholine (ACh) was also injected every 10 min; and leukotriene (LT) $D_4$, U-46619 or neurokinin (NK) A was injected with a 20-min interval between each dose. Test compounds were administered when the magnitude of bronchoconstriction induced by mediators became steady. As ET-1 did not produce steady state $\Delta$PIP, ET-1-induced bronchoconstriction was measured by the method of Konzett and Rössler (16) as described above. For the i.v. and i.d. examination, test compounds were administered 1 and 5 min before the mediator injection, respectively. When drugs were administered i.t., an aerosolized solution of the test compound was inhaled for 1 min and 1 min after this, histamine was injected. With the exception of ET-1, effects of the test compounds were expressed as inhibition of the $\Delta$PIP induced by each mediator. Inhibition of ET-1-induced bronchoconstriction is assessed by a comparison between the $\Delta$VOFV of the compound-treated group and that of the non-treated (control) group. The inhibitory activity of the drugs was expressed as the dose (i.v. and i.d. study) and concentration (i.t.) that suppressed mediator-induced bronchoconstriction by 50% ($ED_{50}$ and $EC_{50}$, respectively).

*Materials*

Ovalbumin, gallamine triethiodide and theophylline (Sigma, St. Louis, MO, USA); Freund's complete adjuvant (Difco, Detroit, MI, USA); histamine 2HCl (histamine) and acetylcholine (Nacalai Tesque, Kyoto); neurokinin A, U-46619 and leukotriene $D_4$ (Funakoshi, Tokyo); endothelin-1 (human; Peptide Institute Inc., Osaka); and sodium pentobarbital (Dinabot, North Chicago, IL, USA), were purchased. T-440 was synthesized by the Lead Optimization Research Laboratory, Tanabe Seiyaku (Yodogawa-ku, Osaka).

T-440 and theophylline were dissolved in saline or distilled water with 2% Tween 80, in the i.v. or i.d. studies, respectively, and were dissolved in saline with 1% propylene glycol in the i.t. study. These solvents had no significant effect on antigen- and chemical mediator-induced bronchoconstrictions.

*Statistics*

All data are expressed as means or means±S.E.M. $ED_{50}$ and $EC_{50}$ values were obtained by graphic evalu-

ation of the half maximal effect from each dose- and concentration-response curve. Statistical analyses were performed by ANOVA and Bonferroni's method, and $P<0.05$ was considered to be statistically significant.

## RESULTS

### *Antigen-induced bronchoconstriction in anesthetized guinea pigs*

Intravenous injection of OA (30 μg/kg) produced a marked increase in ΔVOFV (82±3.9% of maximum ΔVOFV) lasting for more than 10 min in passively sensitized guinea pigs (Fig. 1A). T-440 given i.v. produced a dose-dependent inhibition of antigen-induced bronchoconstrictions. Significant inhibition of bronchoconstriction was obtained at ≥3 mg/kg and the $ED_{50}$ was estimated to be 2.3 mg/kg. Theophylline also produced dose-dependent inhibition of bronchoconstriction ($ED_{50}$ = 15 mg/kg) (Fig. 1A). T-440 was 6.5 times as effective as theophylline on inhibition of antigen-induced bronchoconstriction. As shown in Fig. 1B, a significant increase in heart rate was observed by i.v. administration of theophylline at ≥10 mg/kg. T-440 also increased heart rate significantly at 3 mg/kg, whereas the effect of this drug did not show dose-dependency and was less potent than that of theophylline.

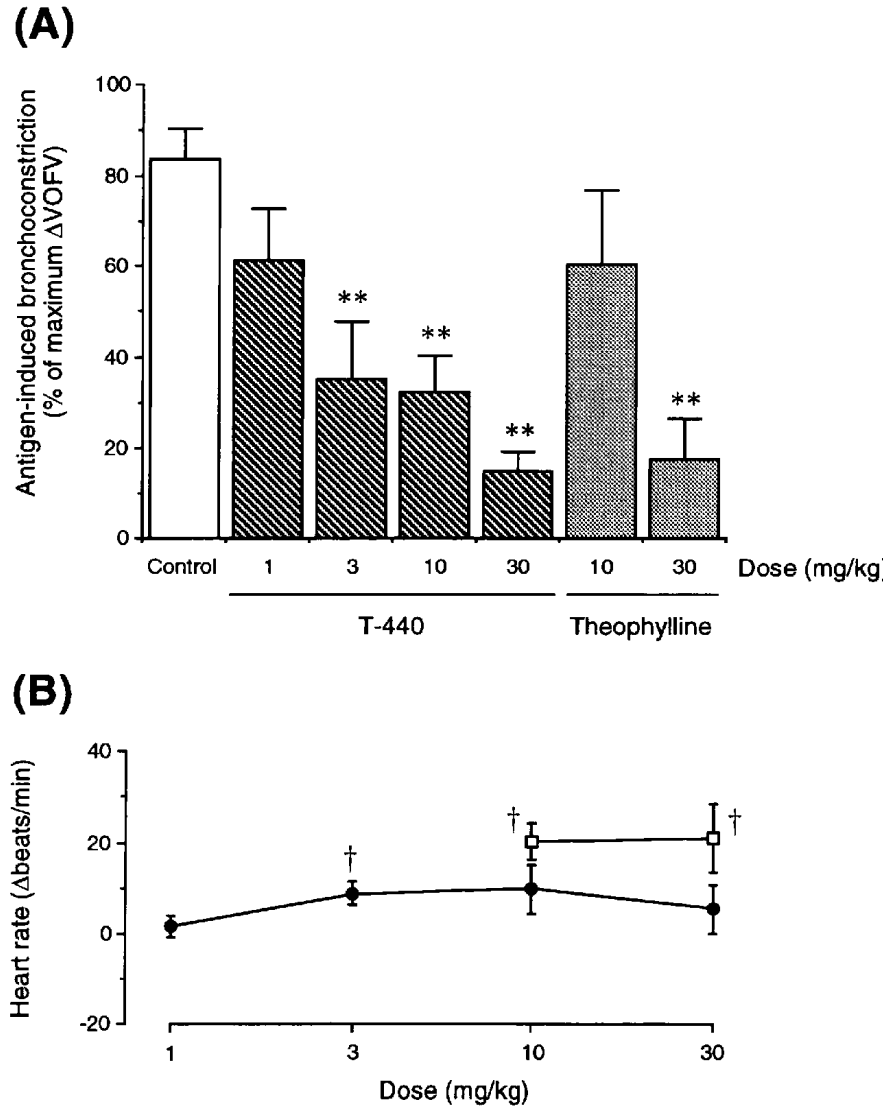


**Fig. 1.** Effects of T-440 and theophylline on antigen-induced bronchoconstriction (A) and heart rate (B) in passively sensitized guinea pigs. T-440 and theophylline were administered i.v. 2 min before the ovalbumin challenge. Each point represents a mean±S.E.M. (N=4–9). **$P<0.01$, compared with the control (Bonferroni's method); †$P<0.05$, compared with the value before administration (Student's *t*-test). ΔVOFV: increase in ventilation overflow volume.

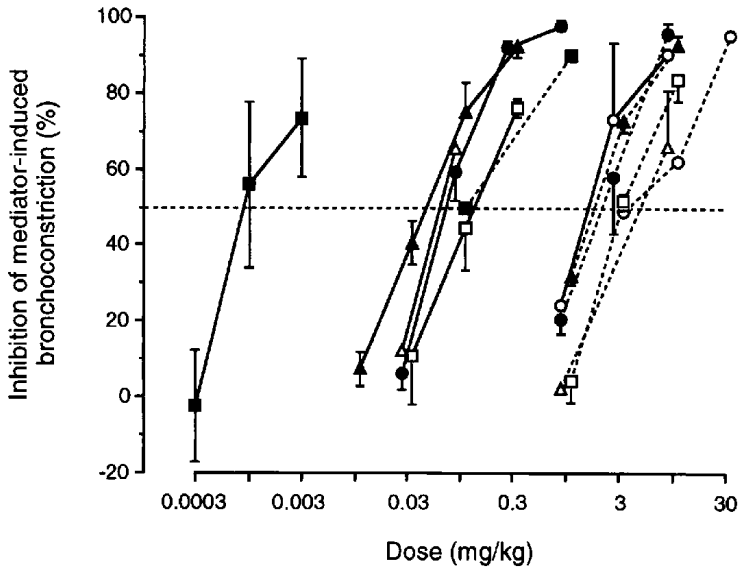


**Fig. 2.** Effects of intravenous administration of T-440 (solid line) and theophylline (dotted line) on chemical mediator-induced bronchoconstriction in anesthetized guinea pigs. Effects on bronchoconstriction induced by histamine (●), leukotriene $D_4$ (■), U-46619 (▲), acetylcholine (○) and neurokinin A (□) are expressed as the inhibition of the increase in pulmonary inflation pressure (ΔPIP). Inhibition of endothelin-1-induced bronchoconstriction (△) was assessed by comparing the increase in ventilation overflow volume (ΔVOFV) of the compound-treated group with that of the non-treated (control) group. Test compounds were administered i.v. 1 min before the injection of each mediator. Each point represents a mean±S.E.M. (N=2–6).

*Chemical mediator-induced bronchoconstriction in anesthetized guinea pigs*

Histamine (2 μg/kg), $LTD_4$ (0.1 μg/kg), U-46619 (1 μg/kg), ACh (7.5 μg/kg) and NKA (3 μg/kg) all increased PIP similarly (25–30 $cmH_2O$) by intravenous injection. ET-1 (300 pmol/kg) produced an increase in ΔVOFV (91±3.2% of maximum ΔVOFV). These bronchoconstrictions were transient and lasted for less than 5 min. Intravenous injection of T-440 and theophylline inhibited these mediator-induced bronchoconstrictions dose-dependently (Fig. 2) and $ED_{50}$ values were estimated to be 0.89–1800 and 100–5600 μg/kg, respectively (Table 1). The most potent suppressions were produced by both drugs on the bronchoconstriction induced by $LTD_4$, whereas the effects against ACh were very weak. The antispasmolytic effects of T-440 were 1.8–110 times as potent as those of theophylline (Table 1).

Intraduodenal administration of T-440 and theophylline inhibited histamine-induced bronchoconstriction in dose-dependent manners (Fig. 3). The effect of T-440 ($ED_{50}$=1.6 mg/kg) was 3.3-fold stronger than that of theophylline (5.3 mg/kg). Treatment with T-440 at 3 mg/kg still significantly inhibited bronchoconstriction at 120 min after its administration, whereas the effect of theophylline (10 mg/kg) disappeared by 60 min (Fig. 3).

Intratracheal administration of T-440 and theophylline by inhalation for 1 min also inhibited histamine-induced bronchoconstriction in concentration-dependent manners (Fig. 4). $EC_{50}$ values were estimated to be 0.49 and >30 mg/ml, respectively. The effects of both compounds reached maxima at 1 min after administration and rapidly disappeared (Fig. 4).

**Table 1.** Effects of intravenously-injected T-440 and theophylline on chemical mediator-induced bronchoconstriction in anesthetized guinea pigs

| Mediators | Inhibition of bronchoconstriction $ED_{50}$ (μg/kg) | | Potency ratio (Theophylline =1) |
|---|---|---|---|
| | T-440 | Theophylline | |
| Histamine | 81 | 2400 | 30 |
| Leukotriene $D_4$ | 0.89 | 100 | 110 |
| U-46619 | 42 | 1600 | 38 |
| Acetylcholine | 1800 | 3300 | 1.8 |
| Neurokinin A | 120 | 2900 | 24 |
| Endothelin-1 | 70 | 5600 | 80 |

The inhibitory effect of each test compound was represented by $ED_{50}$, calculated from the dose-response examination (mean of 2–6 animals at each dose, Fig. 2). Bronchoconstriction induced by endothelin-1 and other mediators were obtained as the increase in ventilation overflow volume (ΔVOFV) and the increase in pulmonary inflation pressure (ΔPIP), respectively (see Materials and Methods). Relative effect of T-440 to theophylline was represented by the potency ratio calculated from the $ED_{50}$ of each compound.

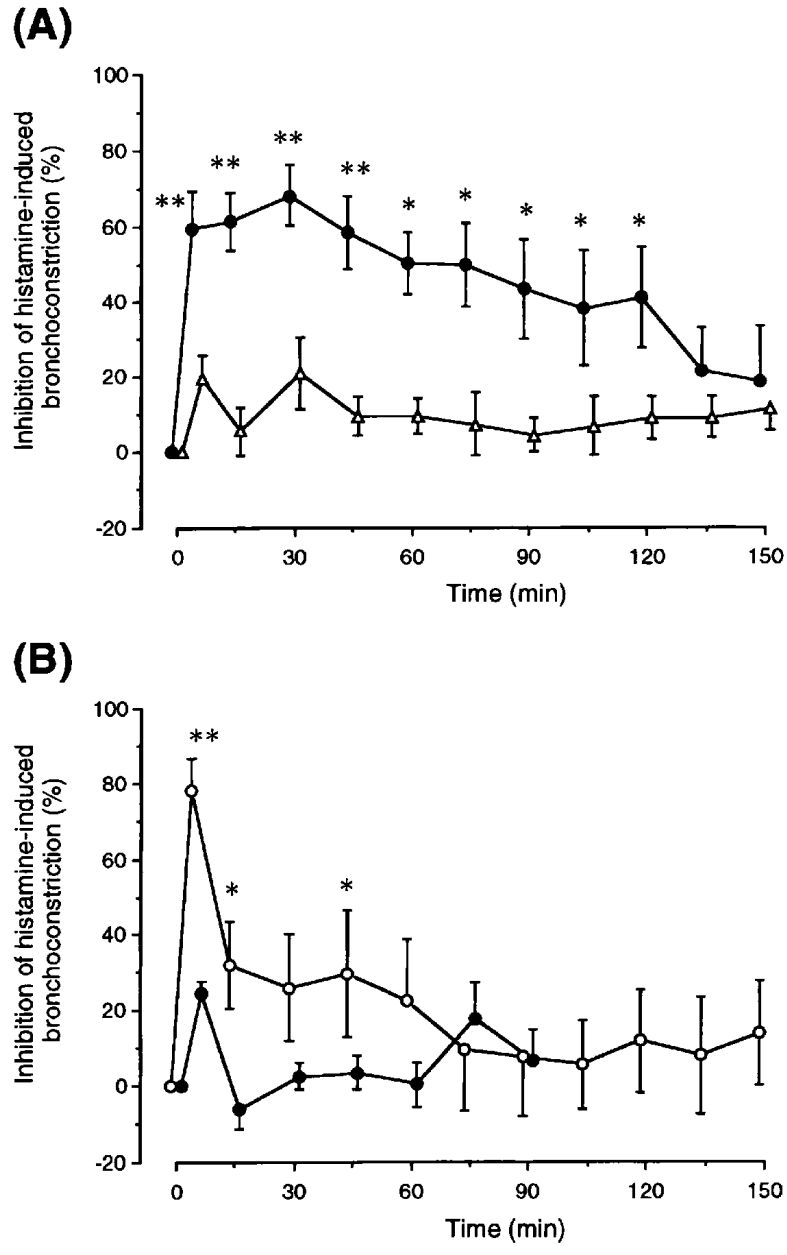


**Fig. 3.** Effects of intraduodenal administration of T-440 (A) and theophylline (B) on histamine-induced bronchoconstriction in anesthetized guinea pigs. Test compounds were administered i.d. 5 min before histamine injection. △, 1 mg/kg; ●, 3 mg/kg; ○, 10 mg/kg. Each point represents a mean±S.E.M. (N=4-6). *P<0.05, **P<0.01, compared with time 0 (Bonferroni's method).

## DISCUSSION

The novel selective PDE IV inhibitor T-440 clearly suppressed antigen-induced bronchoconstriction in passively-sensitized guinea pigs (Fig. 1). Antigen-induced contraction of the airway smooth muscle is mainly caused by bronchoactive substances produced by inflammatory cells such as mast cells (4) and additionally by some tissues such as airway epithelial cells (17) and nervous systems (18). For this reason, we next examined the effect of T-440 on bronchoconstriction induced by histamine, $LTD_4$, U-46619, ACh, NKA and ET-1. Almost all of these spasmogenic substances produced potent and transient bronchoconstrictions as described previously (4, 19-21), and the magnitude of those bronchoconstrictions became steady after several injections of mediators. However, ET-1 did not produce steady state $\Delta$PIP. Although we can not fully explain this discrepancy,

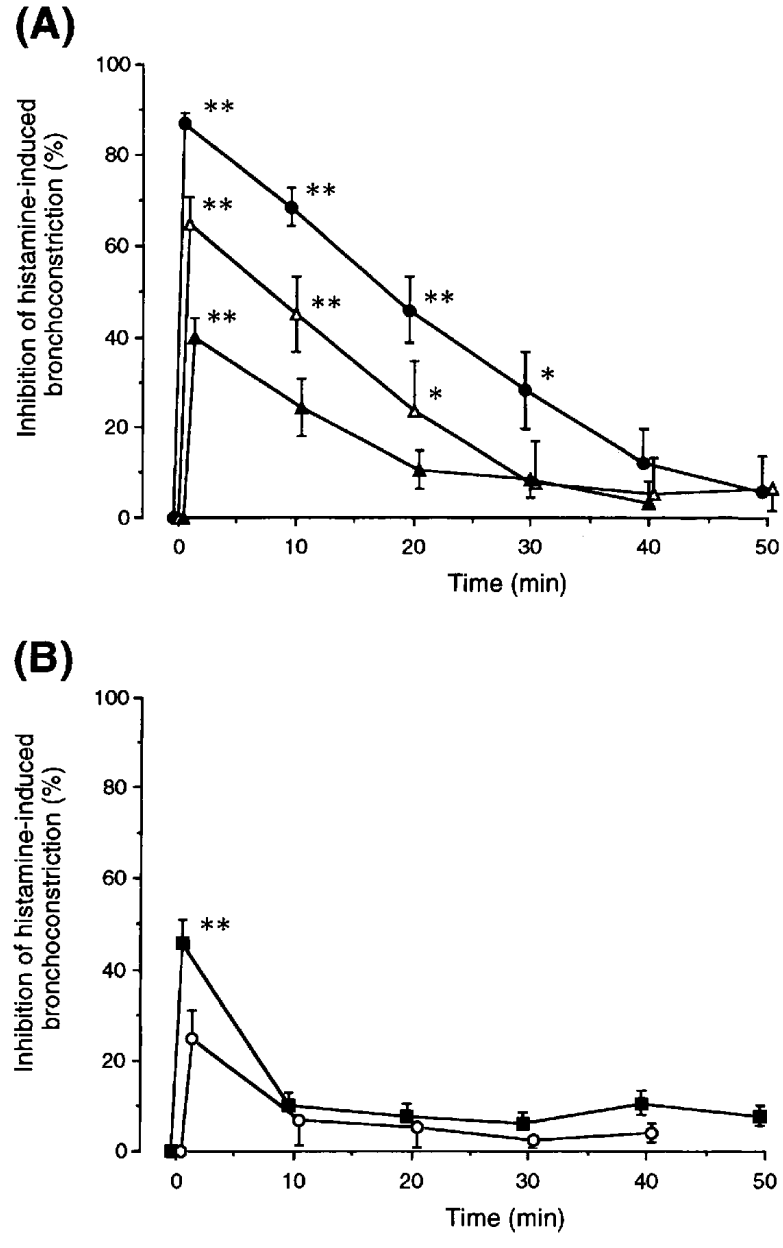


**Fig. 4.** Effects of intratracheal administration of T-440 (A) and theophylline (B) on histamine-induced bronchoconstriction in anesthetized guinea pigs. One minute before histamine injection, aerosolized solution of test compounds was administered i.t. for 1 min. ▲, 0.3 mg/ml; △, 1 mg/ml; ●, 3 mg/ml; ○, 10 mg/ml; ■, 30 mg/ml. Each point represents a mean±S.E.M. (N=5-6). *P<0.05, **P<0.01, compared with time 0 (Bonferroni's method).

Macquin-Mavier et al. (20) reported that intravenous injection of ET-1 produced not only bronchoconstriction but also potent contraction of pulmonary artery smooth muscle. This fact indicates that the repeated injection of this mediator may reduce the diameter of the pulmonary artery and consequently, may produce a situation where ET-1 itself can hardly reach the bronchopulmonary region. This forced us to examine the effect on ET-1-induced bronchoconstriction by measurement of ΔVOFV and comparison with the control group. Even though this method was different from that used for the detection of other mediator-induced bronchoconstrictions, this difference may be negligible, because we have found no significant difference between the effects of the test compounds as determined by monitoring the inhibition of ΔPIP and ΔVOFV in histamine-induced bronchoconstriction (data not shown).

Intravenous administration of T-440 inhibited these

mediator-induced bronchoconstrictions dose-dependently (Fig. 2). Our previous data showed that T-440 selectively inhibited the activity of PDE IV purified from the guinea pig lung (15). PDE IV hydrolyzes cAMP that mediates airway smooth muscle relaxation (14), and other PDE IV inhibitors were also reported to show bronchodilator activity (22). For example, Underwood et al. (23) demonstrated that a selective PDE IV inhibitor, rolipram, attenuated histamine- and $LTD_4$-induced bronchoconstrictions in guinea pigs. Therefore, T-440 seems to suppress chemical mediator-induced bronchoconstriction via PDE IV inhibition and subsequent increase in intracellular cAMP. The suppression of various mediator-induced bronchoconstrictions by T-440 may relate to its prevention of anaphylactic bronchospasm.

The potencies of T-440 to inhibit bronchoconstrictions induced by chemical mediators were quite different from each other, although almost all the magnitudes of bronchoconstrictions were the same level. The effect of T-440 to inhibit bronchoconstriction induced by $LTD_4$ ($ED_{50}$ $=0.89\ \mu g/kg$, i.v.) was 2000 times as potent as that induced by ACh ($ED_{50}=1.8$ mg/kg, i.v.) (Table 1). Rasmussen et al. (24) reported that cholinergic agonists inhibited the activity of adenylate cyclase and subsequently decreased the intracellular cAMP level. This fact may result in low sensitivity to PDE inhibition and relate to a relatively weak effect of T-440 on ACh-induced bronchoconstriction. On the other hand, the potent effect of T-440 on $LTD_4$-induced bronchoconstriction can not be explained by the antagonism at the receptor site, because we have confirmed that $LTD_4$ binding to its specific receptor was not antagonized by T-440 at concentrations up to 10 $\mu$M, in vitro (unpublished data). In contrast with ACh, $LTD_4$ was reported to have no effect on cAMP formation in the airway smooth muscle (25). Further examination of the sensitivity of T-440 to the $LTD_4$-induced bronchoconstriction will be needed.

The efficacy of T-440 in the suppression of histamine-induced bronchoconstriction was demonstrated by multiple administration routes, including i.v., i.d. and i.t. (Figs. 2–4). Administration of a medical drug by an oral route is a most general form of therapy. In addition, treatment of respiratory diseases with drugs by the i.t. route can produce a more rapid and selective effect. T-440, which showed the anti-spasmolytic activity by both administration routes, seems to be convenient for the treatment of airway disorders.

All the effects of T-440 were compared with those of theophylline. Although theophylline also attenuated antigen- and mediator-induced bronchoconstrictions, all the effects of theophylline were less potent than those of T-440. The inhibitory effect of T-440 on histamine-induced bronchoconstriction was 29 times stronger than that of theophylline by i.v. administration (Table 1), whereas it was only 3.3 times stronger by the i.d. route (Fig. 3). This discrepancy may be explained by a difference of bioavailability. Although the absorptivity of T-440 is unknown, theophylline is one of the most easy compounds to be absorbed through the digestive mucosa and the absolute bioavailability of theophylline was nearly 100% (26).

Otherwise, T-440 was $>60$ times as effective as theophylline when administered i.t. (Fig. 4). This ineffectiveness of theophylline may relate to the lack of an i.t.-medication form of this agent clinically.

The increase in heart rate, which is an expected side effect of a PDE inhibitor, was observed by i.v. administration of T-440 (Fig. 1). This effect was statistically significant at 3 mg/kg, but was very weak. These results are supported by our previous data that the effect of T-440 to inhibit PDE III purified from guinea pig heart was 1000 times less potent than that to inhibit PDE IV from the lung (15). In addition to PDE IV, PDE III was also reported to regulate the intracellular cAMP level of the airway smooth muscle, and PDE III inhibition also produced bronchodilation (22). However, T-440 did not cause any potent increase in heart rate even at doses at which antigen-induced bronchoconstriction was almost completely suppressed (Fig. 1). This result clearly demonstrates that T-440 produced a sufficient anti-spasmolytic effect via predominant inhibition of PDE IV, without affecting PDE III. Theophylline with non-selectivity to PDE III/IV produced a potent increase in heart rate even at the dose that did not produce obvious attenuation of bronchoconstriction (Fig. 1).

In conclusion, T-440 inhibited bronchoconstrictions induced by antigen and chemical mediators, and it was effective by i.v., i.d. and i.t. administration routes. All of these effects of this drug were more potent than those of theophylline. A selective PDE IV inhibitor like T-440 may be an effective drug for the management of an airway obstructive disease such as asthma.

*Acknowledgments*

We gratefully acknowledge Dr. Kazuaki Naito for reviewing this manuscript. We thank Ms. Hiromi Isobata and Ms. Kaoru Hashimoto for technical assistance. We also thank Dr. Kazuo Matsumoto and Dr. Tameo Iwasaki for their encouragement.

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