Effect of Tranilast on Endothelin-Induced Bronchoconstriction in Guinea Pigs

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Tranilast, an anti-asthmatic drug with anti-allergic properties, inhibited endothelin (ET)-induced asthmatic like respiratory obstruction in guinea pigs when administered orally at 200 mg/kg 1 h prior to the intravenous injection of ET. ET also caused a bronchoconstriction detected as an increase in inflation pressure in Konzett-Rössler apparatus. Tranilast (200 mg/kg, *p.o.* 1 h before ET) inhibited ET-induced increased inflation pressure. ET-induced bronchoconstriction detected as an increase in inflation pressure was clearly inhibited by the administration of indomethacin (used as a reference drug) at doses of 1 and 5 mg/kg 30 min prior to onset of the reaction. In addition to the above *in vivo* experiments, tranilast at concentrations between 10^{-5} and 10^{-4} g/ml inhibited ET-induced contraction of isolated guinea pig tracheal muscle, whereas indomethacin did not affect this *in vitro* response. In order to elucidate the inhibitory mechanism of tranilast on ET-induced bronchoconstriction, the effects in Ca^{2+} -free medium and on ET-induced histamine and prostaglandin $\text{F}_{2\alpha}$ release from tracheal muscle were investigated. Consequently, tranilast did not affect ET-induced contraction of guinea pig tracheal muscle in Ca^{2+} -free Tyrode's solution and ET induced neither histamine nor $\text{PGF}_{2\alpha}$ release. These results suggest that tranilast inhibits ET-induced bronchoconstriction by inhibiting the ET-induced calcium influx into tracheal muscle.

Keywords — tranilast; endothelin; bronchoconstriction; guinea pig; indomethacin; asthma

Introduction

The potent vasoconstrictor peptide endothelin (ET) has been reported to be one of the most potent *in vitro* constrictors of guinea pig trachea and human bronchial smooth muscle known to date.^{1,2)} Additionally, Macquin-Mavier, *et al.*³⁾ reported that ET causes bronchoconstriction *in vivo* in guinea pigs. They also reported that ET-induced airway muscle constriction is mediated by cyclooxygenase metabolites and modulated by the autonomic nervous system. Because of its potent bronchoconstricting activity, the possible role of ET in physiological and pathological processes is investigated. Recently, Watanabe *et al.*⁴⁾ reported the elevation of ET concentration during asthmatic attack in human. They suggest the pathological role of ET in asthma.

The present study was therefore conducted to determine the effect of tranilast, a well known anti-asthmatic agent with anti-allergic properties^{5,6)} on ET-induced bronchoconstriction in guinea pigs.

Materials and Methods

Materials — Human ET 1 was purchased

from Peptide Institute Inc. (Osaka, Japan). Tranilast was kindly supplied by Kissei Pharmaceutical Co. Ltd. (Matsumoto, Japan). Indomethacin was kindly supplied by Sumitomo Pharmaceutical Co. Ltd. (Osaka, Japan). Human ET 1 was dissolved in saline and diluted with saline to an appropriate concentration. Tranilast and indomethacin were suspended in 0.5% carboxymethyl cellulose saline solution. In *in vitro* experiments, the concentration of the drugs were expressed as g/ml. When their concentrations were expressed on a molar basis, 10^{-5} g/ml of tranilast and indomethacin are equal to 3×10^{-5} and 2.8×10^{-5} M, respectively.

Animals — All experiments were performed on male Hartley guinea pigs weighing 250 to 350 g (Japan SLC. Co. Ltd., Shizuoka, Japan).

Bronchoconstriction *in Vivo* — Bronchoconstriction was measured in two ways. The first measurement was carried out according to previously described methods.⁷⁾ In brief, guinea pigs were anesthetized with pentobarbitone sodium (37.5 mg/kg, *i.p.*). The trachea was cannulated and a polyethylene catheter was placed in the right external jugular vein for ET administration. The tracheal cannula was then connected to a transducer coupled with a multi-purpose

monitoring apparatus (Nihon Kodan Ind. Co., RM-150 type and RM-25 type) to simultaneously record respiratory rate and volume. At the same time, the ratio between expiration and inspiration time (expiration/inspiration ratio) was automatically calculated by computer (PC-9800, NEC, Tokyo, Japan) from the respiratory curve pattern.

The other measurement was performed according to the technique of Konzett and Rössler.⁸⁾ Guinea pigs were anesthetized with urethane (1.5 g/kg, i.p.). The trachea was cannulated and the jugular vein cannulated. The tracheal cannula was connected to a constant volume respirator (New England Inst., Mass. U.S.A.) and the animal artificially ventilated at a constant volume of 5 ml at a frequency of 70 cycles/min. Changes in inflation pressure at constant airflow were measured by a pressure transducer (UGO Basel, Milano, Italy) connected to the side-arm of the tracheal cannula and expressed as a percentage of the maximum increase in inflation achieved by ligating the trachea at the end of the experiment. In each experiment, tranilast was administered *p.o.* 1 h prior to the injection of ET. Indomethacin was administered i.p. 30 min before ET. From the preliminary experiments, each drug appeared to have the most effective anti-allergic or anti-inflammatory action by administering at the above conditions.

Contractile Studies of Tracheal Smooth Muscle *in Vitro* — Guinea pigs were exsanguinated. The trachea was excised and excess tissue trimmed. The open trachea was cut in 16 segments. Four segments were tied together to form a chain and placed in an organ bath containing Tyrode's solution. Changes in tone of the preparation with 0.5 g initial resting tension were recorded isotonically (MEC, ME-4013, World Medical Co. Ltd., Nagoya Japan). Tissues were exposed to the drugs for 5 or 30 min prior to the addition of ET. Contractile responses were expressed as a percentage of 10^{-7} g/ml carbachol-induced contraction.

To examine the effect of the agents on ET-induced contraction in Ca^{2+} -free Tyrode's medium, the organ bath was rinsed twice with 0.1N HCl solution after recording of the contrac-

tion caused by 10^{-7} g/ml carbachol. Both organ bath and tracheal preparations were then washed 10 times with Ca^{2+} -free Tyrode's solution. Exactly 10 min after the last washing, ET was added to the organ bath to measure the contractile response.

ET-Induced Release of Histamine and Prostaglandin $\text{F}_{2\alpha}$ ($\text{PGF}_{2\alpha}$) from Guinea Pig Tracheal Muscle — Tracheal muscle was separated from cartilage. A 300 mg portion of muscle was incubated in 10 nM ET in 2 ml Tyrode's solution at 37 °C for 15 min. The amount of histamine in the supernatant was measured by the method of May *et al.*⁹⁾ The concentration of $\text{PGF}_{2\alpha}$ was analyzed by radioimmunoassay according to the method of Dray *et al.*¹⁰⁾ As a comparative experiment, the antigen-induced

Fig. 1. Effect of Tranilast on ET-Induced Respiratory Obstructions in Guinea Pigs

Each point represents the mean of 6 to 7 experiments. The standard error is not shown for clarity, but was less than 12.5% of mean at all points. Tranilast was administered *p.o.* 60 min prior to the injection of ET. *a)* $p < 0.05$, *b)* $p < 0.01$.

○, control; ●, tranilast 100 mg/kg; △, tranilast 200 mg/kg.

Fig. 2. Effect of Tranilast on ET-Induced Increase in Airway Resistance in Guinea Pigs

Tranilast was administered *p.o.* 60 min prior to injection of endothelin. Each group consisted of 6 animals. Standard error is not shown for clarity but was less than 11.8% of mean at all points. *a)* $p < 0.05$, *b)* $p < 0.01$.

○, control; ●, tranilast 100 mg/kg; △, tranilast 200 mg/kg.

Fig. 3. Effect of Indomethacin on ET-Induced Increase in Airway Resistance in Guinea Pigs

Indomethacin was administered *i.p.* 30 min prior to injection of endothelin. Each group consisted of 6 animals. Standard error is not shown for clarity but was less than 13.2% of mean at all point. *a)* $p < 0.05$, *b)* $p < 0.01$.

○, control; ●, indomethacin (1 mg/kg); △, indomethacin (5 mg/kg).

histamine and $\text{PGF}_{2\alpha}$ release from sensitized guinea pig tracheal muscle was measured. Guinea pigs were sensitized by two *i.p.* injections of ovalbumin (1 mg) at 5 d intervals. Two weeks later the last injection, tracheal muscle was isolated and challenged with antigen (10^{-5} g/ml) by a method similar to that described above.

Statistical Analysis — Statistical analysis was performed using Dunnet multiple comparison.¹¹⁾

Results

Effect of Tranilast on ET-Induced Bronchoconstriction *in Vivo*

Intravenous injection of ET (1 nmol/kg) induced a decrease in respiratory rate and tidal volume and an increase in expiration/inspiration ratio (Fig. 1). These changes are similar to those in experimental asthma. Tranilast at 200 mg/kg inhibited the decrease in tidal volume and the increase in expiration/inspiration ratio induced by ET-1. In the Konzett-Rössler method, bronchoconstriction was reached a maximum 30 seconds after ET injection and gradually decreased thereafter (Fig. 2). Tranilast at 200 mg/kg in-

Fig. 4. Effect of Tranilast (A) and Indomethacin (B) on ET-Induced Constriction of Isolated Guinea Pig Trachea. Each point represents 6 to 8 experiments. *a)* $p < 0.05$, *b)* $p < 0.01$.

(A) ○, control; ●, 10^{-5} g/ml; △, 10^{-4} g/ml. (B) ○, control; ●, 10^{-6} g/ml; △, 10^{-5} g/ml.

Fig. 5. Effect of Calcium-Free Tyrode's Solution on ET-Induced Constriction of Isolated Guinea Pig Tracheal Muscle with and without Tranilast

Each experiment consisted of 4 to 5 experiments. (A) Normal, (B) Ca-free. *a*) $p < 0.01$.

○, control; ●, tranilast (10^{-4} g/ml).

hibited early bronchoconstriction (between 30 s and 1 min).

Indomethacin at doses of 1 and 5 mg/kg completely inhibited ET-induced bronchoconstriction (Fig. 3).

Effect of Tranilast on ET-Induced Contraction of Tracheal Muscle *in Vitro*

On the isolated guinea pig trachea, ET elicited a dose-dependent contraction over concentrations of 2×10^{-10} to 2×10^{-8} M. ET-induced contraction was inhibited by pretreatment with tranilast (10^{-5} and 10^{-4} g/ml) but not by indomethacin (10^{-6} and 10^{-5} g/ml) (Fig. 4). To confirm the effects of drugs, the period of pretreatment with each drug was expanded up to 30 min. The pharmacological activity and potency of each drug was not changed from the

results obtained from the experiments done by 5 min pretreatment. In order to investigate the inhibitory mechanism of tranilast on ET-induced contraction of tracheal muscle the effect in Ca^{2+} -free Tyrode's solution was studied. As shown in Fig. 5, the contractile response to ET in Ca^{2+} -free Tyrode's solution was not effected by tranilast at a concentration of 10^{-4} g/ml. **ET-Induced Histamine and $\text{PGF}_{2\alpha}$ Release from Tracheal Muscle**

ET caused no detectable release of histamine or $\text{PGF}_{2\alpha}$ from tracheal muscle, whereas antigen-antibody reaction caused significant release of histamine and $\text{PGF}_{2\alpha}$ (Table I).

Discussion

These results indicate the efficacy of tranilast in inhibiting ET-induced bronchoconstriction in guinea pigs and the possible inhibitory mechanism. This seems to be related to the inhibition of ET-induced calcium influx into tracheal muscle.

In a previous study we reported that tranilast inhibited LTD_4 -induced contraction of isolated guinea pig tracheal muscle but did not affect histamine-induced responses.¹²⁾ Apparently, compared to histamine, the contractile response to LTD_4 developed slowly and persisted for a long time after agonist removal. Findlay *et al.*¹³⁾ reported that the different contractile responses to LTD_4 and histamine reflect differences in calcium mobilization during agonist-stimulated contraction. ET shows similar contraction induction as LTD_4 . Contraction of guinea pig tracheal muscle by ET persisted long after washing to remove the agonist. The contractile mechanisms of LTD_4 and ET appear similar. It is interesting that tranilast is able to inhibit both

TABLE I. Effect of ET and Antigen-antibody Reaction on the Release of Histamine and $\text{PGF}_{2\alpha}$ from Guinea Pig Tracheal Muscle

	Histamine (ng/g tissue weight)	$\text{PGF}_{2\alpha}$ (ng/g tissue weight)
Spontaneous	1.4 ± 0.26	13.2 ± 0.52
ET	1.3 ± 0.45	11.8 ± 0.43
Ag-Ab	$36.8 \pm 1.84^{a)}$	$72.2 \pm 7.21^{a)}$

Each value represents mean S.E.M. of 5 to 7 experiments. *a*) $p < 0.01$.

LTD₄- and ET-induced contraction but not that caused by histamine. If ET and LTD₄ share a common contractile mechanism tranilast may affect it in the same manner. Concerning the role of Ca²⁺ for ET-induced contraction of smooth muscle, some investigators¹⁴⁻¹⁶⁾ reported that ET-induced vasoconstriction is related to both incorporation of extracellular Ca²⁺ and release of Ca²⁺ from intracellular store sites. The present results suggest the participation of both Ca²⁺ mechanisms for the ET-induced contraction of tracheal muscle *in vitro*. In Ca²⁺ free Tyrode's solution, ET still caused a contraction of tracheal muscle whereas the magnitude of contraction is lower than that produced in normal Tyrode's solution. It is interesting that tranilast inhibited the ET-induced contraction of tracheal muscle in normal Tyrode's solution, but not in Ca²⁺ free medium. It may indicate no direct effect of tranilast on intracellular Ca²⁺ movement. The inhibitory mechanism of tranilast seems to be related to the inhibition of incorporation of extracellular Ca²⁺ due to ET. However, our present results are not direct evidence to support the above idea. Further experiments to examine the effect of tranilast on ET-induced incorporation of ⁴⁵Ca into tracheal smooth muscle cells are necessary. In our previous experiments, we showed that tranilast inhibited the incorporation of ⁴⁵Ca into rat peritoneal mast cells stimulated by the antigen-antibody reaction (unpublished data). Now, in order to determine the effect of tranilast on Ca²⁺ movement in activated cells, some experiments studying the effect of tranilast on ⁴⁵Ca incorporation into mast cells, macrophages and smooth muscle cells stimulated by calcium ionophore A23187, compound 48/80 and ET are in progress. We will discuss the above data in the near future.

Regarding the participation of eicosanoids in ET-induced bronchoconstriction, the present results indicate the inhibitory action of indomethacin on bronchoconstriction *in vivo* but not *in vitro*. Moreover, ET did not generate PGF_{2α} from guinea pig tracheal preparations *in vitro*. With respect to the participation of eicosanoid on ET-induced bronchoconstriction, Battistini *et al.*¹⁷⁾ and Filep *et al.*¹⁸⁾ reported that

ET-stimulates thromboxane A₂ (Tx A₂) generation from tracheal muscle and it may participate in the contraction of tracheal muscle. Advenier *et al.*¹⁹⁾ and Henry *et al.*²⁰⁾ found that indomethacin did not affect ET-induced constriction of guinea pig and human bronchus. The present results confirmed their data. These results suggest that ET-induced bronchoconstriction is mediated by direct activation of Tx A₂ generation and not via prostaglandin generation. Contrary to the above *in vitro* data, indomethacin clearly inhibited the ET-induced bronchoconstriction *in vivo* in the present study and in Macquin-Mavier's study.³⁾ Miura *et al.*²¹⁾ and Rakugi *et al.*²²⁾ found that ET generated eicosanoid from vessel wall cells. ET-induced bronchoconstriction *in vivo* may therefore be mediated by the eicosanoids, *i.e.*, PGF_{2α} and Tx A₂ released from tissues other than tracheal muscle including vessel walls. In addition to the reversal action of indomethacin *in vitro* and *in vivo*, ET showed an additional pharmacological action on the respiratory system without bronchoconstriction *in vivo*. Intravenous injection of ET indicated the prolongation of the expiration/inspiration ratio. This respiratory pattern is similar to asthmatic respiration based on submucosal edema of airway tract. This may suggest that ET acts on airway secretion or pulmonary permeability other than bronchoconstriction. We are now trying to examine the effect of ET on pulmonary circulation. We need further experiments to elucidate the role of ET for respiratory obstruction *in vivo*.

As tranilast has been reported to be a potent inhibitor of histamine release,^{23,24)} the possibility of histamine release due to ET was examined. However, ET did not cause histamine release from guinea pig tracheal muscle.

In conclusion, the present results indicate the efficacy of tranilast in inhibiting ET-induced bronchoconstriction in the guinea pig. The inhibitory mechanism may be related to ET-induced calcium influx into the tracheal muscle.

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