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 \(10^{-9}\) 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 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 PGE\(_{2}\) 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 \(3 \times 10^{-5}\) and \(2.8 \times 10^{-5}\) 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 Koden 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. 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 contractile response 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 F$_{2\alpha}$ (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. The concentration of PGF$_{2\alpha}$ was analyzed by radioimmunoassay according to the method of Dray et al. As a comparative experiment, the antigen-induced contraction was measured.

![Graphs](image)

**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.
Tranilast and Endothelin

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.

histamine and 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. 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).

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.
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 PGF$_{2\alpha}$ Release from Tracheal Muscle**

ET caused no detectable release of histamine or PGF$_{2\alpha}$ from tracheal muscle, whereas antigen-antibody reaction caused significant release of histamine and 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. Apparently, compared to histamine, the contractile response to LTD$_4$ developed slowly and persisted for a long time after agonist removal. Findlay et al. 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>
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<th>TABLE I. Effect of ET and Antigen-antibody Reaction on the Release of Histamine and PGF$_{2\alpha}$ from Guinea Pig Tracheal Muscle</th>
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<td>Histamine (ng/g tissue weight)</td>
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Each value represents mean S.E.M. of 5 to 7 experiments. $^a$) $p<0.01$. 

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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 PGF2α 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 broncus. The present results confirmed their data. These results suggest that ET-induced bronchoconstriction is mediated by direct activation of TxA2 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., PGF2α and TxA2 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.

References


